VOLUME 65 • JUNE 1958 • NUMBER 6

# PATHOLOGY

A Periodical Devoted to General and Experimental Pathology

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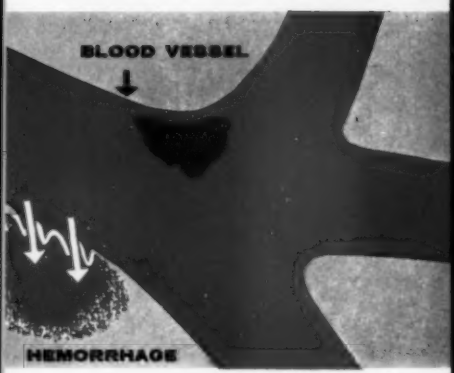
Checks, money orders, and drafts should be made payable to the American Medical Association, 535 North Dearborn Street, Chicago 10.

**AMERICAN MEDICAL ASSOCIATION Publication**

Published monthly by the AMERICAN MEDICAL ASSOCIATION. Editorial and Circulation Offices: 535 North Dearborn Street, Chicago 10, Illinois. Publication Office: Thompson Lane, Box 539, Nashville 1, Tennessee. Change of Address: Notice to the circulation office should state whether or not change is permanent and should include the old address. Six weeks' notice is required to effect a change of address. Second-class mail privileges authorized at Nashville, Tenn., Aug. 6, 1956.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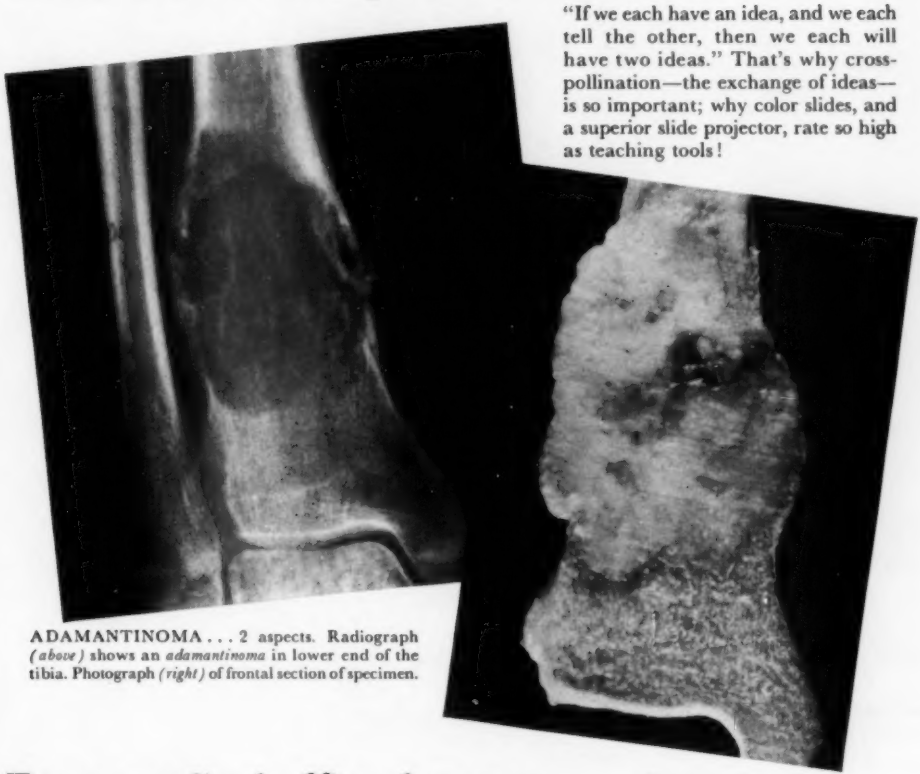

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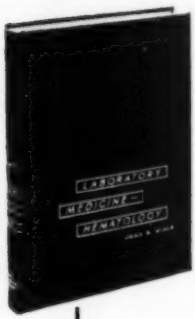

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PATHOLOGY

# In Vivo and in Vitro Cellular Changes Specific for Measles

FRANK E. SHERMAN, M.D., and GISELA RUCKLE, M.D., Pittsburgh

Recorded observations of the pathologic anatomy of fatal measles are few. The scarcity of published material may reflect not only the low mortality but also the natural reluctance of observers to report cases lacking in lesions specific for measles. Such specific lesions are usually absent in cases in which death occurs after the onset of the rash.

The specific cellular changes in measles are concerned with the formation of two types of giant cells. These two types of giant cells will be referred to in this paper as reticuloendothelial giant cells and epithelial giant cells. The eponyms often applied to the reticuloendothelial giant cells, i. e., "Alagna cells," "Finkeldey cells," or "Warthin cells," will not be used.

This report describes autopsy, tissue culture, and virologic studies on the four fatal cases of measles coming to autopsy at Children's Hospital of Pittsburgh during a two-year period. One of these patients, Case 25,* died in the prodromal phase of the disease and showed both the reticuloendothelial and the epithelial, inclusion-bearing, giant cells of measles. Giant cells resembling those of the epithelial type developed in cultures of tissues obtained from this patient at autopsy. As previously reported,[1] virus was recovered from the tissues of this case. The other three patients, Cases 23, 24, and 26, died of secondary bacterial infection after the appearance of the rash. They showed little anatomic evidence of measles and are briefly noted. The histologic characteristics of a single case of Hecht's giant-cell pneumonia are also presented and compared to those of measles.

Submitted for publication Nov. 29, 1957.

Publication Number 139. Department of Pathology, University of Pittsburgh School of Medicine.

From the Departments of Pathology, Children's Hospital of Pittsburgh and the University of Pittsburgh School of Medicine, and the Virus Research Laboratory, University of Pittsburgh School of Medicine. Dr. Ruckle's present address is as follows: Dr. Gisela Ruckle-Enders, Hygiene-Institut und Medizinal-Untersuchungsamt, der Universität Marburg/Lahn, Germany.

* The case numbers used here are the same as in Ruckle's "Studies with Measles Virus."[1,2]

## Presentation of Case 25

### *Clinical Summary*

The history of a 21-month-old white girl's fatal illness began in March, 1956, when a number of cases of rubeola occurred in her neighborhood. Her closest contact with this disease was her 4-year-old brother, who developed a typical measles rash on April 26. The course of his disease was in no way unusual, and he had resumed his normal daily routine by May 8. Three other siblings, ages 11, 12, and 15 years, had all recovered from measles years before this outbreak. The patient developed a cough, anorexia, and "watering of the eyes" on Saturday, May 12. The following day her eyes were puffy, and she was given a child's-sized tablet of acetylsalicylic acid, the only medication to this time. On Monday afternoon, May 14, she began to choke on mucus in her throat, became cyanotic, and had a convulsion followed by unconsciousness for an unknown period of time. Her father gave her artificial respiration for five minutes and took her to a local hospital, where an injection of peni-

cillin was given intramuscularly. Her temperature was normal, and her parents were advised to take her home. She was taken to the family physician's office, and he injected two cc. of γ-globulin intramuscularly. Another convulsion occurred in the physician's office. It began with twitching of the lips and tongue, followed by stiffness and then twitching of the right side of the body. She became comatose. Her rectal temperature was 100 F. She was sent to Children's Hospital of Pittsburgh.

Birth and development had been normal. A transient fever in February had been ascribed to teething. Other illnesses had been occasional colds. Her mother, age 36, and her father, age 39, were in good health, as were all siblings. There was no family history of tuberculosis, diabetes, or allergy.

Physical examination on admission revealed a well-developed and well-nourished stuporous child who roused from a sleep-like state only when disturbed. Her skin was clear, and she was well hydrated. No lymph nodes were palpable. The pupils reacted to light. There was some bleeding from a small laceration on the right buccal mucosa. Koplik spots were not present. The tonsils were hyperemic, and pale exudate lay in the crypts. The neck was supple. The heart rate was 140 per minute. No murmurs were heard. The lungs were clear to percussion and auscultation. The abdomen was soft and easily palpated, but neither liver, spleen, nor other masses could be felt.

Spinal fluid was clear and contained 3 cells (lymphocytes) per cubic millimeter. The Pandy test revealed a trace of globulin. Culture for bacteria failed to grow. The hemoglobin was 11.5 gm. per 100 cc.; RBC, 4,400,000, and WBC, 17,500, with 50% polymorphonuclear leukocytes, 5% metamyelocytes, 42% lymphocytes, and 3% monocytes. Culture of the nose taken on admission yielded Staphylococcus pyogenes var. albus, Hemophilus influenza, and yeast. The throat culture grew Neisseria catarrhalis, a viridans Streptococcus and Escherichia coli.

Her admission rectal temperature of 102.4 F fell to 100 F after she had been placed on a water mattress and had taken 150 cc. of water by mouth. Her only medication the first day was phenobarbital sodium, 1 grain (0.065 gm.) intramuscularly. She was restless during the night and coughed frequently.

Deterioration of her clinical course was rapid and progressive the following morning. It was marked by uncontrollable convulsions, respiratory obstruction, respiratory paresis, and gallop rhythm. Therapeutic measures included phenobarbital and paraldehyde, penicillin, oxygen tent, pharyngeal aspiration of mucus, and finally hydrocortisone, levarterenol (Levophed), and artificial respiration. Death from respiratory paralysis occurred at 3 p. m., May 15, 1956.

### *Material Obtained for Viral Studies at Autopsy*

The autopsy was begun one hour after death, and as it progressed slices of lung, brain, spleen, whole cervical and mesenteric lymph nodes, the right kidney, heart blood, and spinal fluid were collected with sterile precautions and submitted for viral studies. The viral studies were begun as soon as all of the material was collected.

### *Gross Autopsy Findings*

The only positive findings on external examination were a crusted exudate at the inner canthus of each eye and hyperemia of the pharynx, with a gray membranous exudate on the tonsils. Neither skin rash nor Koplik spots were present; there were no palpable lymph nodes.

The brain was severely edematous and bulged as the dura was cut. It weighed 1050.0 gm. The right frontal lobe was removed for virus study before fixing the remainder in neutral formalin. Coronal sections after fixation revealed no focal lesions.

The lungs together weighed 130.0 gm. The trachea and larger bronchi were filled with tenacious turbid yellow exudate. The lungs were pale and emphysematous, with small dark focal regions of atelectasis. There was no consolidation.

The spleen weighed 30.0 gm. The organ was a little less flabby than normal, and the Malpighian bodies were slightly enlarged. The lymphoid tissue in the intestines and the mesenteric and mediastinal lymph nodes were moderately enlarged.

Other organs were essentially unaltered.

Acute swelling of the brain and bronchial obstruction were the causes of death.

### *Microscopic Studies*

The two types of multinucleated giant cells generally considered specific for measles were well demonstrated in this case. Fused epithelial cells of respiratory mucosa were found in the trachea and bronchi. The reticuloendothelial giant cells of measles were observed in lymph nodes, tonsils, thymus, spleen, and the intestinal lymphoid tissue.

Absence of secondary infection made the lungs in this case especially valuable material for the study of the epithelial giant cells. They were present in large numbers in the trachea and larger bronchi, were scarce in bronchioles, and were absent in alveoli. These giant cells occurred focally and in varying stages of development in

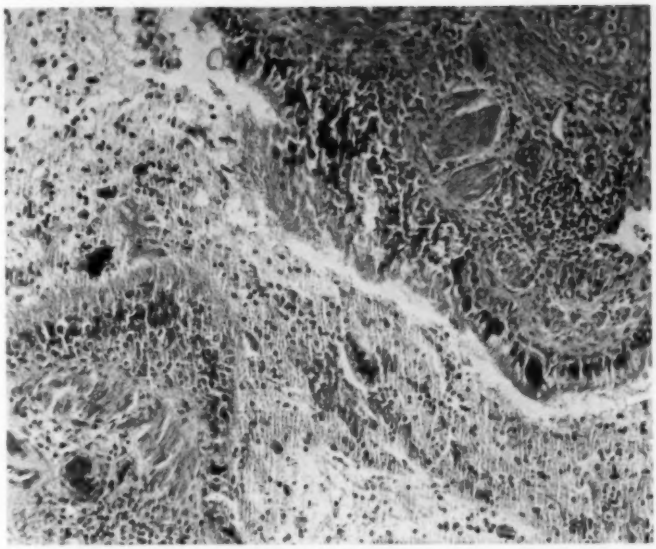

Fig. 1.—Syncytial epithelial giant cells are forming in the bronchial mucosa. Two are lying freely in the lumen. Hematoxylin and eosin; reduced 10% from mag. × 140.

each bronchus (Fig. 1). Judging duration by the severity of degenerative changes, a rough chronology of their evolution could be postulated.

The first variation from normal was the appearance of a fine eosinophilic granularity in the cytoplasm of some 10 to 100 adjacent cells. Disappearance of cell walls accompanied this change.

Coarsening of the cytoplasmic granularity was the next step, which included slight swelling of nuclei. Many intranuclear inclusions appeared at this time. They were amphophilic and generally had a circular or slightly ovoid outline, but a few were crescentic, and some were irregular. A large clear halo separated them from the nuclear membrane where the chromatin was concentrated (Fig. 2).

Further development consisted of increased nuclear basophilia, with shrinking and clumping into a dark lobulated

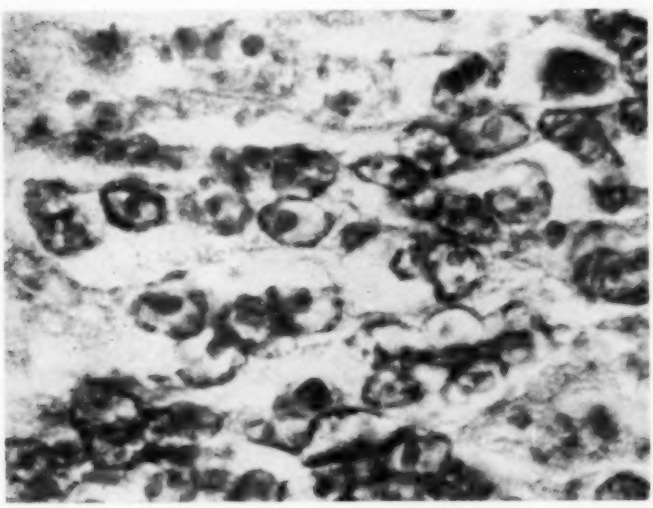

Fig. 2.—Inclusion bodies lie in the pale central portions of this group of nuclei in a syncytial bronchial epithelial cell. Hematoxylin and eosin; reduced 15% from mag. × 1200.

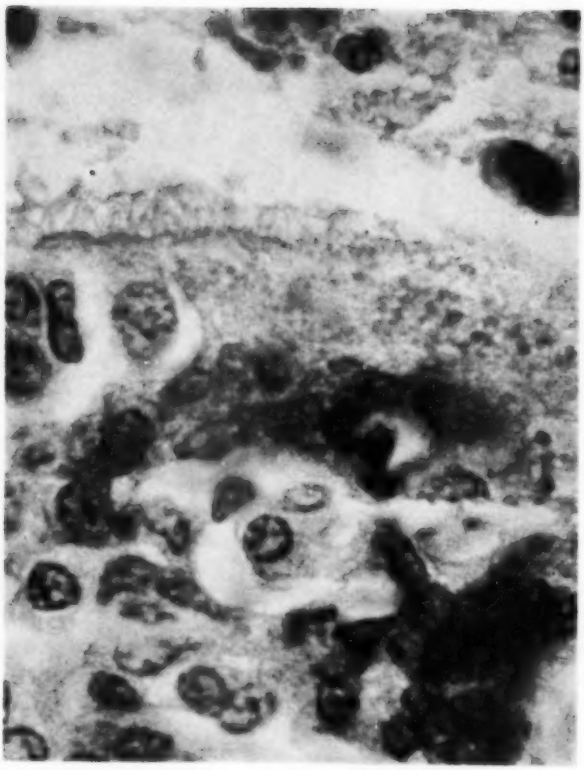

Fig. 3.—Intracytoplasmic granules lie below the cilia in this photomicrograph. Hematoxylin and eosin; × 1200.

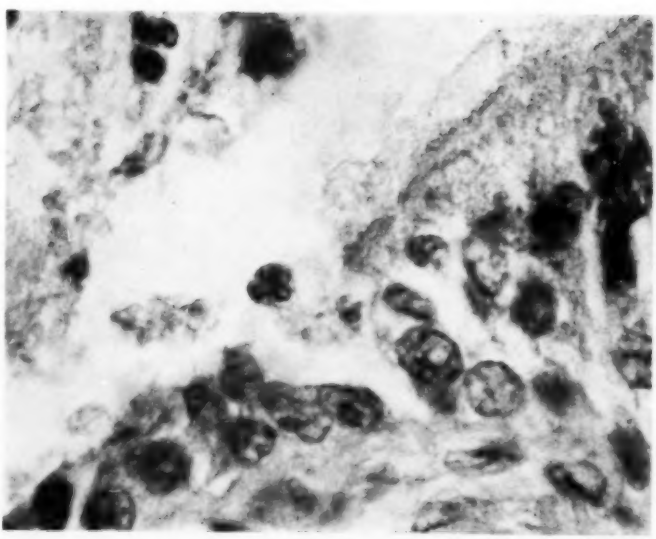

Fig. 4. — Basal cells, seen at lower left of this photomicrograph, remain after sloughing of a giant cell. The giant cell is not included in the photomicrograph. Hematoxylin and eosin; reduced 15% from mag. × 1200.

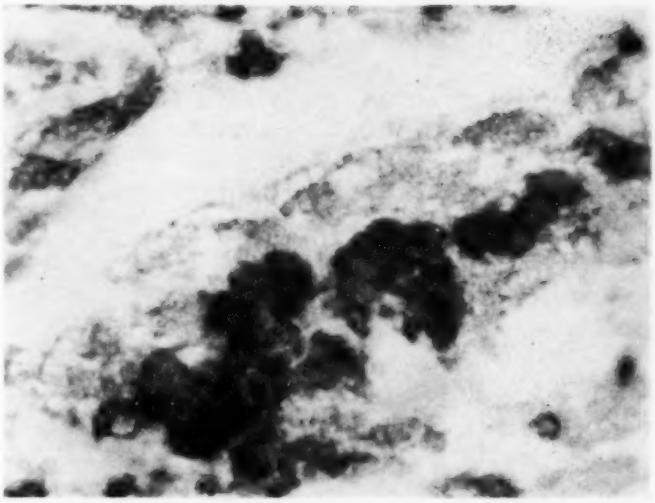

Fig. 5.—This recently sloughed epithelial giant cell is lying freely in the bronchial lumen. Note the coarsely granular cytoplasm and the persistence of the cilia. Hematoxylin and eosin; reduced about 10% from mag. × 1200.

chromatin mass at the base of the syncytial cytoplasm. This clumping was so intense as to render identification of individual nuclei difficult. Intranuclear inclusions were not identifiable at this stage. Cytoplasmic changes also progressed. Some of the larger focal granules developed small clear zones around them. Rarely, these granules attained the size of an erythrocyte (Fig. 3). Most were considerably smaller.

The final step in formation of these giant cells was apparently their separation from the unaffected basal layer of cells so that they came to lie freely in the lumen (Fig. 4). In addition to these epithelial giant cells the bronchial lumens contained macrophages, cellular debris, a few granulocytes, and mucus. The more recently sloughed giant cells were intact, and many maintained cilia. Disintegration, noted in some, consisted of progressive loss of the coarsely granular cytoplasm, so that only the nuclear clumps remained of some giant cells. Occasionally slight intranuclear eosinophilia

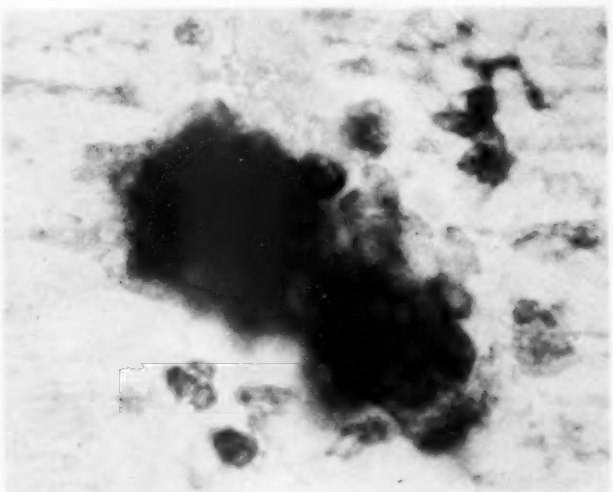

Fig. 6.—This giant cell, also lying in the bronchial lumen, has lost most of its cytoplasm. The nuclei are pale centrally. Hematoxylin and eosin; × 1200.

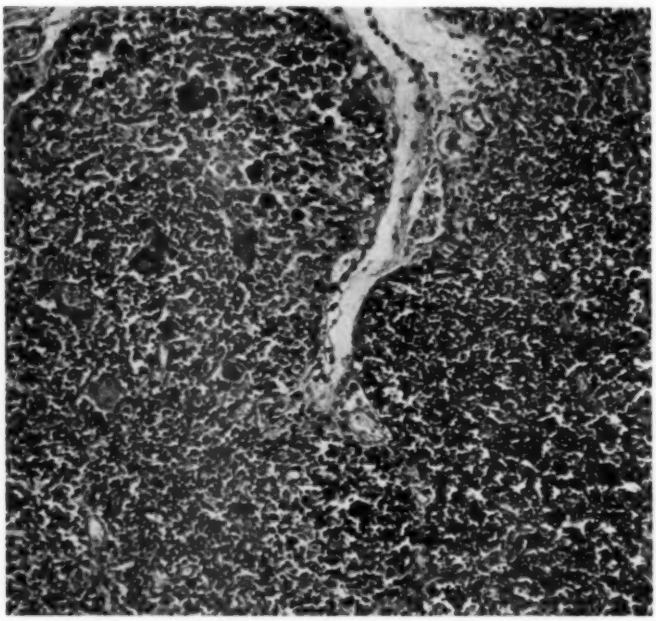

Fig. 7. — Reticuloendothelial giant cells are seen in the thymus. Most are concentrated in upper left of figure. Hematoxylin and eosin; × 140.

was suggestive of inclusions in these nuclear clumps (Figs. 5 and 6).

Mild patchy interstitial pneumonitis involved about one-third of the pulmonary tissue. It consisted of macrophages on and in the alveolar walls. These cells had finely granular eosinophilic cytoplasm, but definite inclusions could not be identified. Accompanying edema was minimal, and hyaline membranes were absent. Clumped nuclei, which resembled the reticuloendothelial giant cell of measles, were frequently seen in the lumens of the pulmonary arteries and rarely seen in alveolar capillaries. However, they could not be definitely differentiated from the postmortem nuclear debris

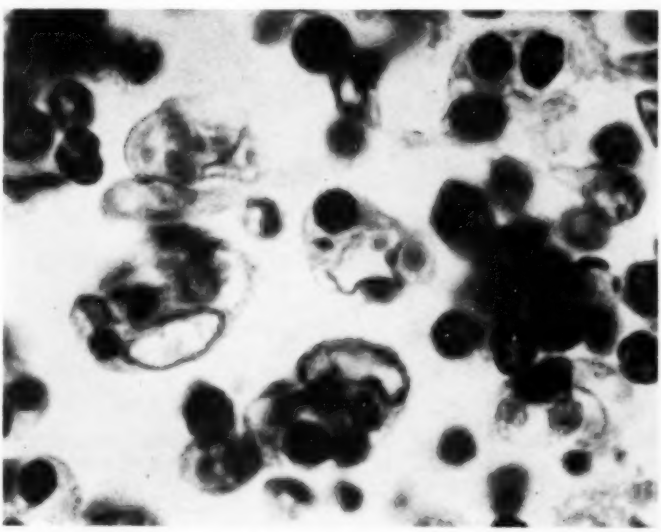

Fig. 8. — Phagocytized nuclei lie within the cytoplasm of macrophages, whose large nuclei are pale centrally. Hematoxylin and eosin; reduced about 5% from mag. × 1200.

that is occasionally observed in routine autopsies and is interpreted by some as megakaryocytes.

Reticuloendothelial giant cells of measles were observed in all lymphoid tissues studied. These included mediastinal, abdominal, and cervical lymph nodes; tonsils; spleen; thymus (Fig. 7), and the lymphoid tissue in the intestines, including the appendix vermiformis. These cells were best studied in the edematous lymph nodes, where they were widely disseminated. The larger cells consisted of 50 or more clumped nuclei with indefinite and exceedingly scanty cytoplasm. In some clumps, individual nuclear membranes could be identified; in others, only lobulated dark basophilic masses were present. The smaller giant cells, containing up to 15 nuclei, were surrounded by a definite cytoplasm and membrane. Origin of some of the smaller giant cells by phagocytosis of small round cells resembling lymphocytes by macrophages was observed (Fig. 8), but when more than about 15 of these nuclei were present in a single macrophage, identification of the macrophage nucleus became impossible. Consequently the larger giant cells could not be proved to be of phagocytic origin. The macrophages were concentrated in the follicles but were also present elsewhere. They had a fairly distinctive granular eosinophilic cytoplasm, giving them an epithelioid appearance. Some cytoplasmic granules were very coarse and had indefinite halos, but their identity as viral inclusions was indefinite. These macrophages had large ovoid nuclei, with pale centers, and condensed peripheral chromatin. No intranuclear inclusions were present. Another feature of the lymph nodes was marked swelling of vascular endothelial nuclei without inclusions or granular cytoplasmic change.

There was diffuse acute neuronal degeneration in the brain. Occasional neuronophagia was observed. These neuronal changes were accompanied by edema, hyperemia, and minimal perivascular hemorrhage. Neither inclusions nor exudate were present. Hematoxylin-and-eosin-stained sections of frontal, motor, and occipital cortex; basal ganglia; Ammon's horn; midbrain; pons; medulla, and cerebellum all contained these changes.

### Note on Case 26

Measles rash appeared on the second day of a 5-year-old white girl's illness. Pain in her legs and back appeared on the eighth day, and it ascended to her neck on the ninth day, which was also the day of her death. Nuchal rigidity, Kernig's sign, and the Babinski reaction were present. The extremities had become flaccid before death.

At autopsy, the lesions considered responsible for death were acute swelling of the brain and acute mucopurulent bronchitis. A minimal leptomeningeal exudate was composed of lymphocytes and macrophages. No significant neuronal degeneration was seen.

The only specific giant cells in any of the sections were in the urinary bladder. Patches of superficial mucosal cells had formed syncytial masses containing two to six nuclei (Fig. 9). These cells had a swollen finely granular eosinophilic cytoplasm, with small perinuclear vacuoles. The nuclei were round and slightly swollen and had peripheral concentration of chromatin. Some contained a central amphophilic rounded body representing an inclusion of possible viral origin.

Samples of various regions of the brain were obtained at autopsy. They were stored at −20 C for several days and then transferred to a −70 C environment. Virus studies were begun approximately one year later.

### Note on Case 23

A 22-month-old white girl died on the 11th day of her disease. A typical measles rash had appeared on the seventh day, and progressive respiratory difficulty had begun on the ninth day. Periods of apnea and cardiac standstill marked her clinical course.

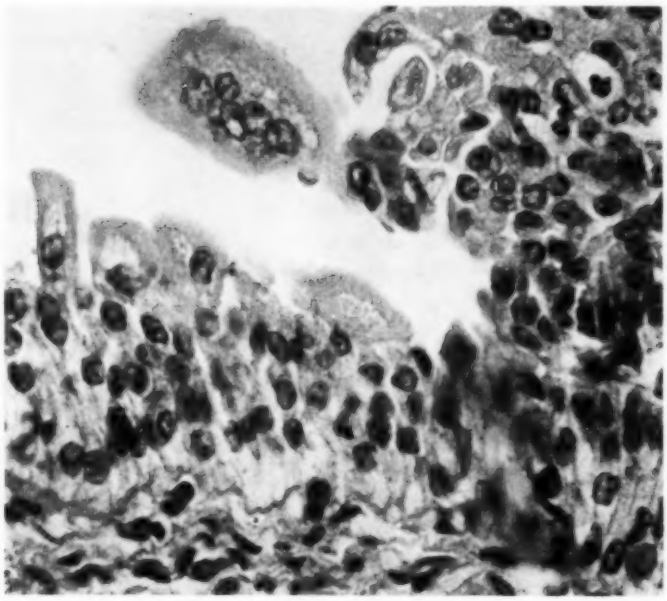

Fig. 9.—Syncytial giant cells in mucosa of urinary bladder. Hematoxylin and eosin; reduced about 5% from mag. × 480.

The autopsy findings were those of acute necrotizing and obstructive bronchitis. No giant cells specific for measles were present in any of the organs.

The autopsy was begun two hours after death, and samples of brain, lymph nodes, spleen, skin, and heart blood were obtained. These specimens were stored at −20 C for 10 days before virus studies were begun.

### Note on Case 24

A 16-month-old white girl developed a measles rash on the fourth day of her illness and died on the seventh day. Her course was marked by spells of progressively severer respiratory difficulty and cyanosis.

The major autopsy findings were those of obstructive mucopurulent bronchitis. Specific giant cells were absent in all sections, including those of skin, which demonstrated microscopic changes of the measles rash.

Spinal fluid, heart blood, and pharyngeal mucus were collected one hour after death. Samples of brain and lung were obtained at autopsy, which began five hours after death. Viral studies were begun immediately.

### Virological Observations

Virologic studies of these cases have been previously reported in detail,[1,2] and the following is a summary of the results.

Tissue extracts were prepared from the autopsy material that had been stored for various lengths of time at different temperatures. Body fluids and the different tissue extracts were inoculated into pregrown cultures of human amnion and human and monkey renal cells. In Case 25 the additional procedure of trypsinizing and culturing the tissues was employed. No measles virus could be isolated from tissue extracts and body fluids tested from Cases 23, 24, and 26. Serum samples which were available in Case 23 and Case 24 were tested for the presence of antibodies for measles. Neutralizing as well as complement-fixing antibodies were detectable at early postinfection levels.[2]

In Case 25, measles virus was not isolated from blood or spinal fluid. Of the tissue extracts, which were prepared from lung, kidney, spleen, liver, mesenteric lymph node, and brain, only the lung extract induced cytopathic changes characteristic for

measles virus on inoculation into human amnion cultures. Trypsinized cultures were prepared from lung, kidney, spleen, brain, and cervical and mesenteric lymph nodes in Case 25. The various cultures were maintained from 4 to 12 weeks. A considerable variation in growth potential and cell morphology with increasing time of incubation was observed. The epithelial-like cells in cultures of lung, spleen, and cervical lymph node were eventually replaced by fibroblastic elements. The kidney cultures remained throughout as epithelial-like cells, whereas cells in mesenteric lymph node and brain cultures started and remained fibroblastic. Cytopathic changes compatible with the presence of measles virus were observed in the cultures of lung, spleen, and cervical lymph node as long as epithelial-like cells were present. The viral agent responsible for the cytopathogenic effect in these cultures was present in the nutrient fluid for some time and could be identified as measles virus. The cytopathogenic changes observed in cells of the kidney cultures characterized by huge eosinophilic cytoplasmic inclusions was not associated with the production of infectious measles virus. No cytopathic changes became evident in mesenteric or brain cultures, and no measles virus was isolated from them.

Trypsinized lung cultures from Case 25 achieved optimal growth by the 10th day after preparation. Epithelial-like cells predominated until the 12th day, when fibroblastic-like cells began to take over. In the 5- to 10-day-old cultures groups of 50 to 100 nuclei with small inclusions were embedded in syncytial cytoplasmic masses containing numerous eosinophilic bodies surrounded by halos (Figs. 10 and 11). In the 12- to 17-day-old cultures fibroblasts surrounded the giant-cell areas. The inclusions in the nuclei of the giant cells now stained brightly eosinophilic, had become larger, and were surrounded by clear halos. In the 20- to 30-day-old cultures the spindle-shaped cells were predominant. Forty days after the preparation of the cultures hardly any giant cells remained. Multiplication of the virus was demonstrated by titration of samples of nutrient fluid. The peak of infectivity was reached in 12-day-old cultures. The disappearance of epithelial-like cells was accompanied by diminution of characteristic cytopathogenic effect and final absence of infectivity.

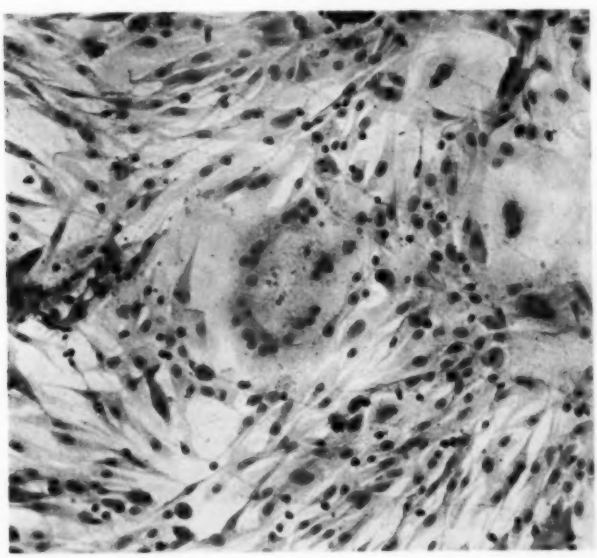

Fig. 10.—A general view of the lung culture at 14 days reveals a syncytial giant cell in the center of the field. Hematoxylin and eosin; × 140.

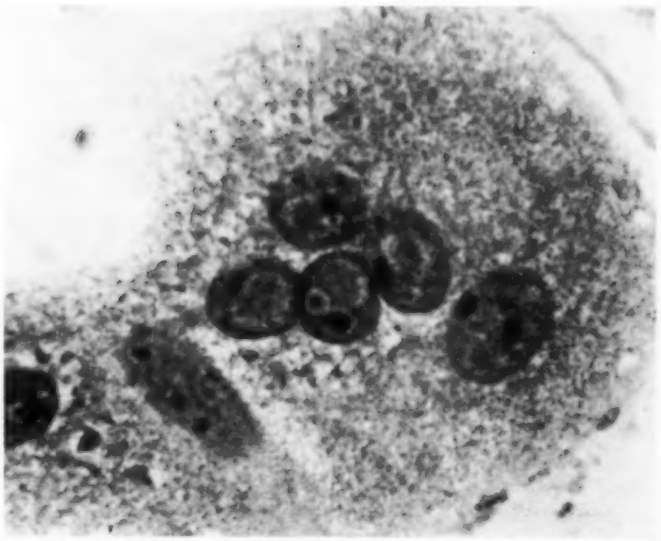

Fig. 11.—Large intranuclear and granular cytoplasmic inclusions are illustrated in this view of a syncytial giant cell in a culture of pulmonary tissue grown 14 days. Hematoxylin and eosin; reduced about 15% from mag. × 1200.

The serum sample obtained from Case 25 contained very low levels of neutralizing antibodies for measles. It is assumed that these antibodies were passively derived from the administration of γ-globulin 24 hours before death, since antibodies are usually absent in the early infectious stage of measles.[2]

## Comment

*Giant Cells of Measles.*—Formation of multinucleated giant cells is the most striking and constant finding in tissues infected with measles virus. This change occurs both in tissue culture and in vivo. In the human being, cells of the reticuloendothelial system and various epithelial cells are transformed into multinucleated giant forms. In direct cultures of organs from our Case 25, the giant cells all resembled those of epithelial type, whether derived from epithelial or from lymphoid organs.

*Giant Cells of Reticuloendothelial Origin.* The giant cells in lymphoid structures are well established as a specific change indicating infection with measles, but their significance in the pathogenesis of this disease has not been defined.

Degeneration is characteristic of the clumped nuclei. Pyknosis, crenation, fragmentation, and the assumption of bizarre shapes have been described by many authors. It seems likely that the crescent-shaped *corps-pseudoprotozoaires* described by Bonenfant[3] are a form of degeneration of these nuclei similar to the crescent-shaped fragments described by Finkeldey.[4]

The origin of these multinucleated giant cells is not definitely known. That they represent an abnormal polynuclear development of stem cells of the lymphocytic series has been suggested.[5] However, this mechanism seems unlikely because the general process in these cells is degenerative and not proliferative. Two modes of genesis are plausible: 1. Finkeldey[4] and others since have suggested that these cells may be formed by an agglutination-like process or a flowing together of lymphocytes. (Such a process appeared in the formation of the epithelial-type giant cells in bronchial epithelium and in tissue cultures.) 2. Phagocytosis also observed by Finkeldey and others was definitely a factor in Case 25, where macrophages containing as many as 15 engulfed nuclei could be identified.

The reticuloendothelial giant cells tend to disappear soon after the appearance of the rash[6] or at the same time that circulating

antibodies become detectable.[2,7] Perhaps neutralization of extracellular virus at this stage stops further formation of these cells. Their disposition is unknown, but the possibility of their gaining entrance to the blood stream is worth considering. The degenerated nuclear material observed in pulmonary arteries and alveolar capillaries in Case 25 and previously described by Milles [8] may represent circulating forms of these cells.

Cells freed from spleen and cervical lymph node by trypsinization of autopsy tissues in Case 25 were cultured and grew well. Parasitized giant cells indistinguishable from those in the lung cultures were formed. These cells are described by the virologist as "epithelial-like," but it is unlikely that any epithelial contamination occurred in the spleen and lymph node cultures. These cells are probably of reticuloendothelial origin and are apparently fertile ground for the growth of measles virus in vitro. In culture, these cells differ considerably from the reticuloendothelial giant cells seen in sections of human material in that they have an abundant cytoplasm and contain both intracytoplasmic and intranuclear inclusion bodies.

*Giant Cells of Epithelial Origin.*—Multinucleated epithelial giant cells consisting of a syncytial mass sometimes containing intracytoplasmic and intranuclear inclusions are specific for measles. They are most often observed in respiratory epithelium and are present in most cases before the appearance of the rash. They are closely related to, if not identical with, the cells in Hecht's giant-cell pneumonia.

These cells have been described by Denton [9] and others.[3,6,8-14] Their morphologic characteristics in various stages of development and degeneration are described under Case 25. Similar cells were observed in the mucosa of the urinary bladder in Case 26, and the presence of these cells has been described in the mucosa of the appendix vermiformis [3] and in the mucosa at the base of the tongue.[14] There is general agreement that these cells arise from syncytial change occurring in two or more adjacent epithelial cells.

The relation of these cells to the clinical stage of the disease is of interest. They can be found in most, if not all, cases in the prodromal stage by examination of smears made from sputum or nasal swabs.[10,13] Autopsies of prodromal measles cases, similar to our Case 25, reported by Semsroth [14] and Stryker [6] revealed epithelial giant cells in the bronchial epithelium. These cells are not found in most instances when death occurs after the rash, but Miles [8] has reported such cells in two cases dying 16 and 17 days after the occurrence of the rash.

Inclusion bodies, as described by Pinkerton et al.,[12] were present in only about 5% of the giant cells in our Case 25, but many more cells contained small closely packed cytoplasmic granules. That such granules may represent virus is suggested by the diffuse fluorescence of the cytoplasm of measles-infected cells in culture observed by Cohen et al.[15] after treatment with fluorescein-labeled measles convalescent serum. Intranuclear inclusions, in Case 25, appeared early in the formation of bronchial epithelial giant cells. After the nuclei had become agglutinated, the only evidence suggesting inclusion bodies was irregular central pale intranuclear regions. In general, the inclusions are apt to be overlooked in paraffin sections because of their variation in size, shape, and staining qualities. However, there is a reasonable similarity between inclusions appearing in tissue sections and those in measles-infected tissue cultures.

*Hecht's Giant-Cell Pneumonia and Measles.*—Pinkerton et al.[12] have recorded the similarity of the morphologic findings in Hecht's disease, distemper, and measles. We have been impressed with the similarity between the epithelial giant cells of measles and the giant cells in a previously unreported case of Hecht's giant-cell pneumonia loaned to us by Dr. R. C. Hamilton, St. Francis Hospital, Pittsburgh. This was a 7-month-old white boy who died 15 days after de-

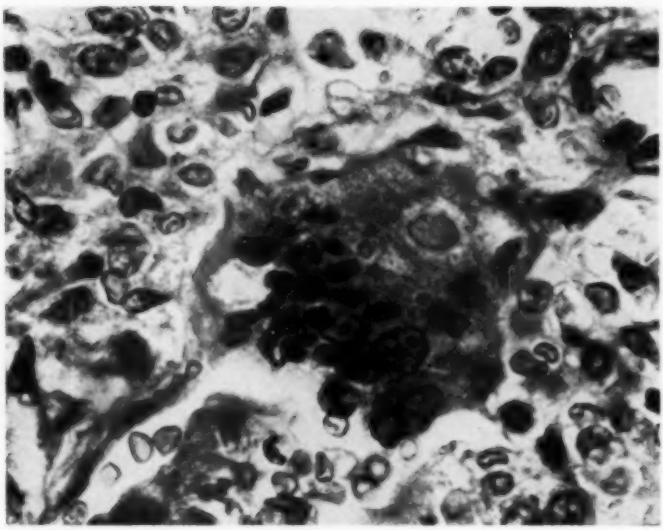

Fig. 12.—Both cytoplasmic and intranuclear inclusions are seen in this photomicrograph of a giant cell in Hecht's disease. Hematoxylin and eosin; reduced 10% from mag. × 1200.

veloping a measles rash. The giant cells in this case differed more in distribution than in cellular characteristics from those in our case of prodromal measles. In this case of Hecht's pneumonia, the giant cells were concentrated in alveolar spaces and terminal bronchioles rather than in the larger bronchi. These cells contained large and sharply defined cytoplasmic inclusion bodies (Fig. 12). They closely resembled the cytopathic change in measles infected tissue culture. The frequent history of preceding measles or exposure to measles and the similarity of cytologic changes suggest that Hecht's pneumonia may be a bizarre form of measles.

## Summary

The autopsy findings in four fatal cases of measles are presented.

Three of these patients died of bacterial bronchitis after the appearance of the rash. Except for the presence of syncytial giant cells in the urinary bladder mucosa in one instance, these cases had no morphologic evidence of measles. This paucity of specific change is typical of measles when examination of tissues is made after the appearance of circulating antibodies and of the rash. Neither was virus demonstrable by inoculation of tissue cultures with tissue extracts obtained at autopsy.

The remaining case died in the prodromal stage without significant secondary bacterial infection. Epithelial and reticuloendothelial giant cells specific for measles were demonstrated in this case. Morphologic observations on the genesis and disintegration of the epithelial giant cells in bronchial mucosa are recorded. Observations of the reticuloendothelial giant cells indicate that phagocytosis is at least one mode of genesis of this cell. Inclusion bodies in the epithelial giant cells of the bronchial mucosa are noted to be similar to those in the syncytial cells seen in tissue cultures. Cytoplasmic granularity is observed in macrophages in lymph nodes and spleen. This granularity is analogous to the earliest morphologic change associated with measles virus infection in tissue culture.

The presence of virus in the spleen, lung, kidney, and lymph nodes but not blood of the patient dying in the prodromal stage was demonstrated by culture of the patient's tissues. Changes typical of measles infection occurred in these cultures, and nutrient fluid from these cultures was capable of inducing typical cytopathic changes when added to pregrown cultures. Of the ex-

tracts tested from lung, kidney, spleen, liver, lymph node, and brain, only the lung extract induced cytopathic change in pregrown tissue cultures.

Attention is called to the morphologic similarities of Hecht's giant-cell pneumonia and measles.

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# Experimental Studies in Metal Cancerigenesis

*IX. Pulmonary Lesions in Guinea Pigs and Rats Exposed to Prolonged Inhalation of Powdered Metallic Nickel*

W. C. HUEPER, M.D., Bethesda, Md.

The successful production of sarcomas affecting bones, connective tissue, nerve tissue, and muscle in rats and rabbits after a parenteral administration of metallic nickel was reported in previous communications.[1,2] While these observations established the fact that metallic nickel is a carcinogen for two species of experimental animals, the cancers were obtained by introducing nickel through a route which is not encountered under occupational conditions of exposure to nickel in man. The tumors produced in experimental animals, therefore, differ in histogenetic type and topographical distribution from those observed in man, which are carcinomas involving the nasal cavity, the paranasal sinuses, and the lungs and developing after inhalation of nickel dust, fumes, and vapors.[3-5]

In studies on occupational carcinogenesis it is important for scientific and medicolegal reasons to produce experimentally cancers of the same histologic type and location in animals by duplicating as closely as practicable the exposure conditions prevailing for man. Hence, rather extensive inhalation experiments with powdered metallic nickel supplied by the International Nickel Company were started several years ago simultaneous with the already reported experiments in which nickel was administered by the parenteral route. Rats, guinea pigs, and mice were used. The results of these investigations are presented in this communication.

Submitted for publication Nov. 25, 1957.

Head, Environmental Cancer Section, National Cancer Institute, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

## Experimental Procedures

The nickel powder used was obtained by precipitating nickel from nickel carbonyl and consisted of more than 99% of pure nickel. The majority of particles had a diameter of 4$\mu$ or less. The powdered metallic nickel was delivered into the dusting chamber in constant and controlled amounts with the aid of a Wright Dust Feed Mechanism, producing a concentration of nickel averaging 15 mg. of nickel per cubic meter of air. The animals were exposed to this atmosphere for 6 hours a day for 4 to 5 days per week for a maximal period of 21 months, at which time all animals used for experimentation had died.

The following types of animals were used: Guinea pigs of the inbred Strain 13, about 3 months old when placed in the dusting chamber, were chosen because they have an average life span exceeding that of other strains. Animals of this particular strain, moreover, had been used in experiments with carcinogenic agents by other investigators.[6,7] Information on the frequency and type of pulmonary tumors in members of this strain under normal and abnormal conditions therefore was available. A total of 42 guinea pigs (32 males, 10 females) was employed. Members of two strains of rats were exposed when from 2 to 3 months old. One hundred rats of the Wistar strain obtained from a commercial breeder evenly divided as to sex and sixty female rats of the Bethesda Black strain made up the test group (total, one hundred sixty rats). Since both strains had been used in other carcinogenic experiments and had been studied in normal control series, adequate information was available as to the occurrence, frequency, topographical distribution, and histologic types of tumors and their precursor conditions for both strains. Similar information was on hand regarding mice of the C57 Black strain, of which 20 female mice about 2 months of age at the start of the experiment were employed. This strain was chosen because the absence of spontaneous lung tumors among its members would have conveyed distinct significance to the appearance of even only a few pulmonary neoplasms in test animals. The only special control group used in this investigation consisted of nine female guinea

TABLE 1.—*Death Distribution of Guinea Pigs, Rats, and Mice Inhaling Metallic Nickel Dust*

| | Exposure, Mo. | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0–6 | 7–12 | 13–15 | 16–18 | 19–21 | 22–24 | Total |
| | Deaths, No. | | | | | | |
| Guinea pigs | 12 | 7 | 20 | 1 | 2 | 0 | 42 |
| Wistar rats | 26 | 38 | 7 | 16 | 11 | 2 | 100 |
| Bethesda rats | 7 | 24 | 26 | 3 | 0 | 0 | 60 |
| $C_{57}$ Black mice | 5 | 12 | 3 | 0 | 0 | 0 | 20 |

pigs which had been used as breeders and which were from 18 to 30 months old when they were killed.

None of the test animals were killed. All of them died while being exposed to the inhalation of nickel dust. Autopsies were performed on all animals. The histologic examination of the various internal organs and tissues of all animals showing any grossly demonstrable pathologic changes included in some rats and guinea pigs the region of the paranasal sinuses. Sections from all organs and tissues examined were stained with hematoxylin and eosin. Sections of the lungs of some rats were incinerated for the demonstration of nickel deposits.

## Postmortem Observations on Experimental Animals

The death distribution of the animals in the various series is presented in Table 1.

### 1. *Gross Observations*

(*a*) Guinea Pigs: Postmortem examinations on guinea pigs showed that edema, hyperemia, and hemorrhages of the lungs not infrequently associated with localized pneumonic indurations were common pathologic reactions. Livers often were grayish-red to pale-yellow-brown in color in many animals, suggesting the presence of fatty infiltration. The adrenals in one guinea pig were considerably enlarged and dark red. Two of the female guinea pigs had serous ovarian cysts measuring about 2 cm. in diameter.

(*b*) Rats: The most frequent pathologic reactions seen at autopsy in rats were fibrinopurulent pleurisy, acute and chronic circumscribed and diffuse pneumonic lesions often of extensive character, pulmonary congestion and edema, and cystic bronchiectases with cheesy contents. Nodular neoplastic reactions involving the mesenteric and retroperitoneal lymph nodes and forming medullary white and hemorrhagic and necrotic masses up to 5 cm. in diameter were found in five Wistar rats. Similar-appearing nodes located in the liver were present in four additional Wistar rats, while one Wistar rat had an encapsulated subcutaneously located breast tumor in the left axillary region and two Wistar rats had dark red nodular enlarged pituitary glands. Only one of the Bethesda Black rats exhibited a medullary white and hemorrhagic nodular mass involving the ileocecal lymph nodes, while a second rat of this strain had a cysticly distended nodular uterus filled with cheesy necrotic matter.

(*c*) Mice: The necropsies performed on the mice showed the presence of white medullary masses in the mesentery and inguinal lymph nodes measuring up to 2 cm. in diameter in a mouse dying one month after the start of the experiment. Similar neoplastic abdominal nodes were observed in a second mouse which died 12 months after this event. The lungs of the great majority of mice

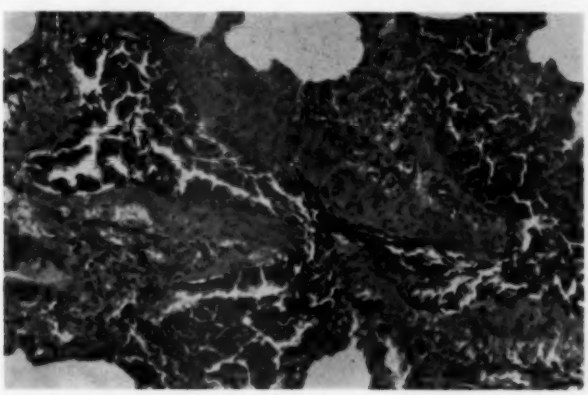

Fig. 1.—Solid epithelial casts of bronchiolar hyperchromatic cells in bronchioli extending into adjacent alveoli; × 143.

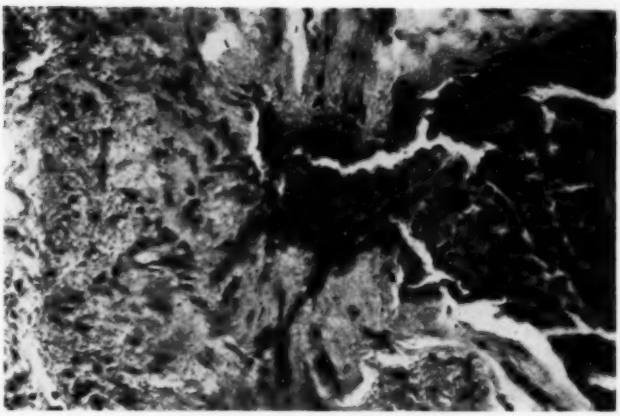

Fig. 2.—Proliferated bronchiolar epithelium filtrating into the wall; × 285.

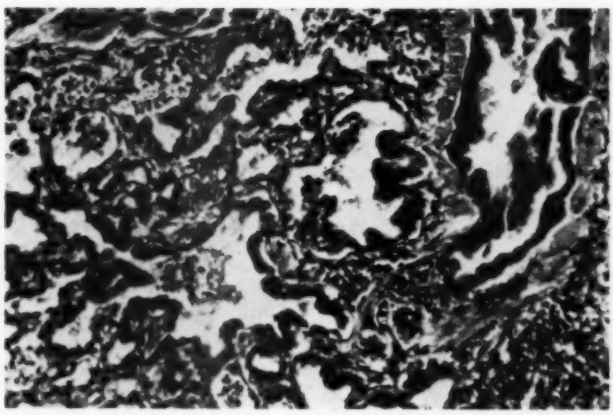

Fig. 3.—Adenomatoid transformation of peribronchiolar alveoli; × 155.

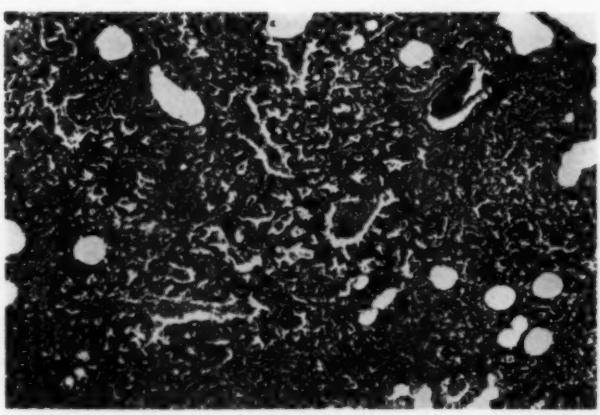

Fig. 4.—Alveolar adenomatosis involving large areas of the pulmonary parenchyma; × 39.

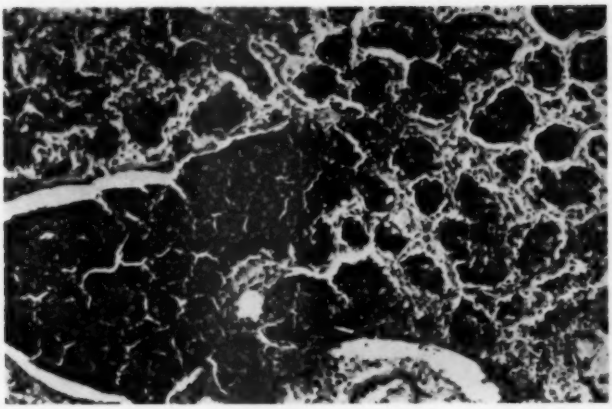

Fig. 5.—Anaplastic alveolar carcinoma of the lung; × 143.

coming to autopsy were hyperemic and hemorrhagic. The livers were congested.

2. *Histologic Observations*

(*a*) Guinea Pigs: The lungs showed, in addition to frequent local to diffuse edema, hyperemia, and hemorrhages, not infrequently combined with circumscribed areas of leukocytic intra-alveolar infiltrations, the following remarkable reactions in the bronchiolar mucosa and in adjacent alveolar areas. The bronchiolar lumens often were filled in their terminal parts by solid casts of slender oval to spindle-shaped and round hyperchromatic small epithelial cells (Fig. 1). Occasionally similar epithelial proliferations were seen in larger bronchi, where the hyperchromatic cells seemed to infiltrate into the bronchial muscular coat (Fig. 2). The proliferating bronchiolar epithelium extended in many areas into the adjacent alveoli, which were then lined by a cuboidal epithelium and assumed an adenomatoid appearance (Fig. 3). Such areas were often rather large and numerous and were not delineated against the surrounding normal alveoli (Fig. 4). Extensive multicentric adenomatoid formations present in some animals produced the impression of an adenomatosis. In some cases a marked irregularity of the formations and a considerable atypia of the cells in both the bronchiolar and the alveolar formations were noticed. Such lesions resembled, in the bronchioli, carcinomas in situ, in the alveoli, miniature adenocarcinomas. In one animal these changes apparently had progressed sufficiently to form a multicentric anaplastic carcinoma (Figs. 5 and 6). Numerous large

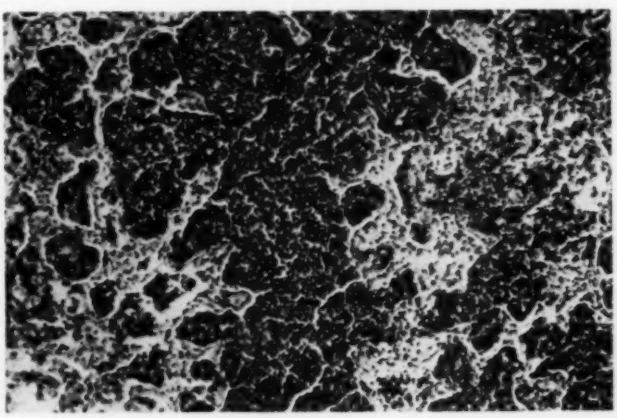

Fig. 6. — Diffuse infiltration of pulmonary alveoli with carcinomatous casts, with partial destruction of intervening pulmonary structures; × 143.

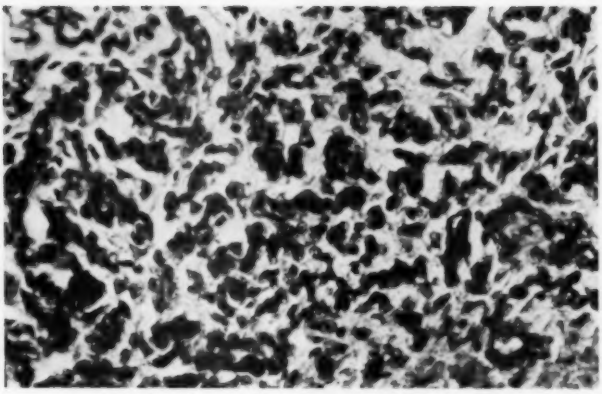

Fig. 7.—Adenomatoid atypical tumor located in the retroperitoneal tissue near the bladder; × 285.

areas in the lung contained alveolar groups solidly filled with small hyperchromatic epithelial cells which invaded the surrounding tissue and extended into the bronchioli. Metastases were not found in the mediastinal lymph nodes or in any other organ or tissue. In a second guinea pig, however, which had numerous adenomatoid formations, a node of adenocarcinomatous tissue was discovered in the lower abdomen near the urinary bladder (Fig. 7). While the primary tumor of the lung was not seen and could have easily been missed at the microscopic examination, since serial sections of the lungs had not been made, there can be little doubt on the basis of its morphology that the abdominal node is of metastatic nature, originating from a pulmonary tumor.

A histologic examination of the region of the nasal sinuses of the guinea pigs was not made, since there was no gross evidence that these structures were abnormal. The particle size of the nickel dust used and the physical form of nickel employed (solid instead of gas or vapor), moreover, did not favor the penetration of nickel into the nasal sinuses. The inadequacy of the available equipment and the general working conditions, on the other hand, prohibited the use of highly toxic nickel carbonyl for chronic experimentation.[15]

In a smaller number of lungs the interalveolar septa and the alveolar lumens were filled with a cellular granulation tissue, often containing giant cells with multiple hyperchromatic nuclei of irregular shape frequently surrounding colorless reflecting rod-shaped inclusions (Fig. 8). The adenomatoid as well as the granulomatous lesions were usually not related to any acute

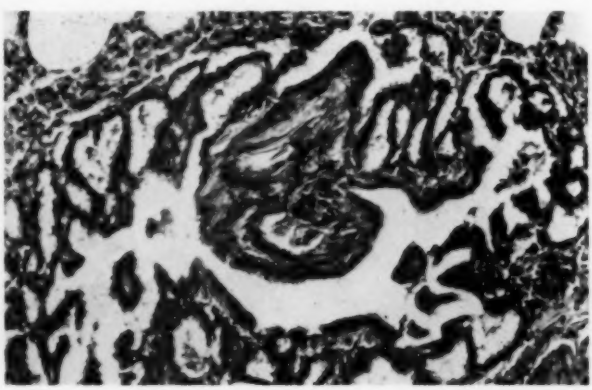

Fig. 8.—Adenomatoid alveolar area in the lung, with fibrous node containing giant cells attached to the surface of reflecting noncolored rod-like inclusions; × 143.

or chronic inflammatory changes present in the lungs. An analysis of the relation between the period of exposure to nickel dust and the extent of the adenomatoid lesions showed that these increased in frequency, extent, and number with the length of the inhalation period. While a few small peribronchial adenomatoid formations were seen in five of the nine old control guinea pigs, none revealed any diffuse adenomatosis such as that observed in almost all test animals.

The majority of the livers exhibited moderate to marked degrees of vacuolation of the liver cells, which sometimes was associated with moderate periportal fibrosis and round-cell infiltrations. Pericentral hyaline hepatic necroses were present in some animals. Calcium casts were occasionally seen in the medullary tubules of the kidney. The other organs were essentially normal.

(*b*) Rats: The lungs of the rats of both strains exhibited in 15 out of a total of 50 rats studied histologically adenomatoid alveolar formations similar to those seen in the guinea pigs. The epithelial lining of these glandular structures seemed to be extensions from the terminal bronchial mucosa. While in some animals these multicentric lesions were associated with chronic and subchronic inflammatory reactions in the surrounding pulmonary tissue, often they occurred without such an associated pathology and were rather numerous. The paranasal sinuses exhibited, as a rule, chronic inflammatory changes often complicated by mucosal ulcers. The findings of the other organs were not remarkable, with the exception of the occurrence of several tumors. The neoplasms originating from and involving mainly the abdominal and mediastinal lymph nodes were large round-cell sarcomas forming metastases in various organs. One had metastasized into the bone marrow of the sternum. The tumors of the livers were fibrosarcomas or spindle-cell sarcomas. The subcutaneously located neoplasm was an adenofibroma, and the two tumors of the hypophysis were basophil adenomas. Since none of them has any obvious relation to the inhalation of nickel, they will not be further considered.

(*c*) Mice: The lungs were hyperemic and often hemorrhagic. None showed any abnormalities of the bronchial mucosa or any adenomatoid formations. The tumors present in the lymph nodes of two mice were lymphosarcomas, which in all probability were unrelated to the exposure to nickel. Since malignant lymphomas occur "spontaneously" among animals of this strain, it is unlikely that neoplastic reactions may plausibly be attributed under such circumstances to a tissue which had no direct contact with the chemical administered.

## Comment

The observations reported indicate that in almost all guinea pigs and in about 50% of the rats of two strains exposed to prolonged inhalation of finely powdered metallic nickel abnormal multicentric adenomatoid formations affecting the alveolar structures and atypical proliferations of the epithelial lining of the terminal bronchioli were present (Table 2).

The four grades are rather arbitrary estimates of adenomatoid involvement of the lungs based on the number and size of adenomatoid formations present. Such data were available on only 37 of the 42 guinea pigs used in the experiment because the organs of 5 animals were too decomposed for permitting a reliable histologic study. In six guinea pigs the intra-alveolar and intrabronchiolar epithelial proliferations assumed a degree of atypia in circumscribed areas approaching the character of microcarcinomas.

TABLE 2.—*Grades of Adenomatoid Proliferations in Lungs of Guinea Pigs After Different Periods of Exposure to Nickel Dust*

| Grade | Exposure, Mo. | |
|---|---|---|
| | 1–6 | 7–21 |
| | Guinea Pigs, No. | |
| 1 & 2 | 7 | 9 |
| 3 & 4 | 1 | 20 |

In one guinea pig an actual anaplastic intra-alveolar carcinoma was found, and in a second one a retroperitoneal node was discovered which in all probability originated from a pulmonary carcinoma. Similar multiple adenomatoid reactions have been reported in guinea pigs after a long-continued total-body exposure to γ-radiation,[7,8] after an intravenous injection of methylcholanthrene,[6] or subsequent to the injection of a diphtheroid bacillus.[9] It is most probable, therefore, that the pulmonary adenomatoid responses in the guinea pigs and rats recorded, as well as, particularly, their carcinomatous sequelae, provide a new additional demonstration of the carcinogenic action of nickel.[1,2] Since these neoplastic and metaplastic responses were obtained by the respiratory introduction of nickel into the lungs, they duplicate to a high degree the conditions which are associated with the appearance of cancers in various parts of the respiratory tract of workers (nasal cavity, nasal sinus, lung) engaged in the smelting of nickel-copper matte in England and Norway.[3,5,10,11] The conclusion, therefore, seems to be justified that they are, like their human counterparts, reactions of the respiratory tissues to the nickel inhaled, which could be demonstrated in the ashed sections prepared from the lungs of some of the rats studied.

The various histologic changes observed in the lungs of guinea pigs and rats make it likely that most of the hyperplastic and metaplastic epithelial proliferations noted had their origin in the terminal bronchioli from where they extended into the adjacent alveoli. Whether or not all of the alveolar-adenomatoid lesions had this bronchiogenic origin, on the other hand, is uncertain, because they were found in areas in which a direct connection with an altered bronchiolus was not apparent. Since, however, serial sections of the lungs were not prepared, this issue must remain undecided. The adenomatoid pulmonary formations resembled in only a very general way those not infrequently seen in mice. They were not nodular in outline and were never papillary in structure but were similar to those described by Norris.[12] They were, moreover, generally distributed throughout the lungs and did not exhibit any preference to the peripheral portions. Because of the frequent absence of concomitant inflammatory reactions in the lungs, the pulmonary adenomatosis cannot be considered a nonspecific response to chronic inflammatory changes, such as those not infrequently noted in the peribronchial tissue of rats suffering from chronic pneumonitis and purulent bronchiectases.[13,14]

The adenomatoid formations seen in the rats which inhaled nickel dust, on the other hand, were often found in areas which were not involved by chronic peribronchial inflammatory changes and which therefore most likely were related in their genesis to the action of inhaled nickel.

## Summary and Conclusions

Practically all guinea pigs and many rats belonging to two strains subjected to long-continued inhalation of finely powdered metallic nickel developed multicentric adenomatoid formations of the alveoli and hyperplastic proliferations of the terminal bronchiolar epithelium.

One guinea pig had an anaplastic intra-alveolar carcinoma, while a second guinea pig, with extensive pulmonary adenomatosis, showed an adenomatoid node resembling in structure the pulmonary lesions in the retroperitoneal space of the lower abdomen.

Both benign and malignant pulmonary neoplastic lesions of guinea pigs and benign hyperplastic adenomatoid proliferations in the lungs of rats are attributable to the nickel dust inhaled and may be considered to be etiologic and organic equivalents of the respiratory neoplastic reactions seen in copper-nickel matte smelter workers.

Nickel has been shown to be a carcinogen to rats, rabbits, and guinea pigs, since it is capable of eliciting sarcomas in rats and rabbits after intraosseous and subcutaneous introduction and of producing adenomatoid, carcinomatoid, and carcinomatous reactions

in the lungs of guinea pigs after inhalation of nickel dust.

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# Carcinogenic Bioassay of Benzo(a)pyrene-Free Fractions of American Shale Oils

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In previous investigations it was shown that crude American shale oil and a few of its high-boiling fractions, like Scottish shale oil and its fractions, are carcinogenic to mice [1] and that benzo(a)pyrene is contained in crude shale oil as well as in a fraction boiling between 400 and 510 C (752 and 950 F) at 760 mm. of Hg.[2] During the fractionation of this shale oil by thermodistillation and multiple chromatographic separation numerous fractions were obtained which did not contain any demonstrable amounts of benzo(a)pyrene and which differed widely in their respective boiling points (from below 380 F to 950 F).[2] These fractions provided material suitable for investigating several additional aspects of scientific and practical importance on the carcinogenicity of shale oils and related hydrocarbon products and were used in the present experiments. It was of interest to ascertain (1) whether or not the totality of the carcinogenic effect exerted by crude shale oil and its various fractions is attributable to the fraction containing benzo(a)pyrene and particularly to its benzo(a)pyrene content only and (2) whether some undetermined chemical components of the crude shale oil might also possess carcinogenic properties and might be present not only in the fraction containing benzo(a)-pyrene but also in fractions having boiling ranges below and above this specific fraction or being obtained by eluents differing from those extracting specifically benzo(a)pyrene from the chromatographic column.

Submitted for publication Nov. 25, 1957.

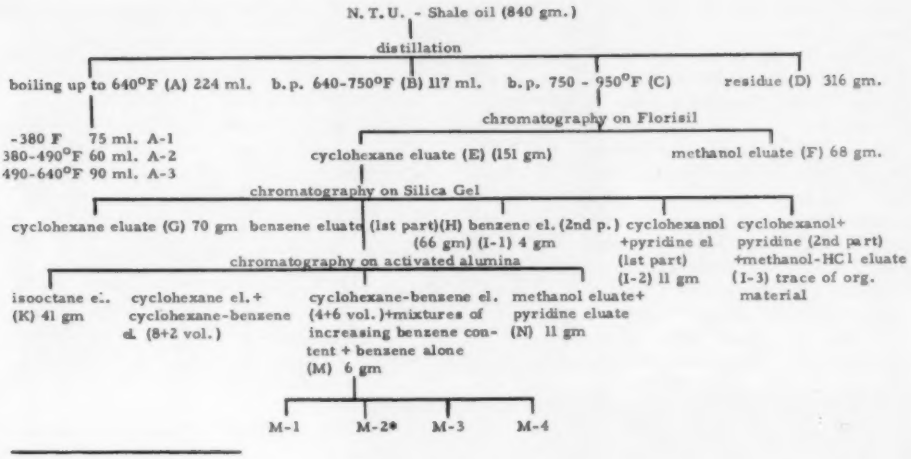
Environmental Cancer Section National Cancer Institute, National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare.

Information on these aspects seemed to be needed for the following reasons. During recent years some investigators have identified the entire carcinogenic action of cigarette tar and of air pollutants with their relative contents of benzo(a)pyrene. The demonstration of this chemical, however, supplies merely a convenient qualitative indicator of the apparent presence of carcinogenic polycyclic aromatic hydrocarbons of various types in such materials and does not provide any information concerning other carcinogens of organic and inorganic nature which might also be present and contribute to the carcinogenic action of such matters. Berenblum and Schoental [3] reported some years ago on the carcinogenic effect of a fraction of coal tar which was free from benzo(a)pyrene. Claims also have been advanced in recent years that fractions of petroleum oils having a boiling point below 700 F are noncarcinogenic.[4] Since observations on several low-boiling fractions of synthetic hydrogenated coal oils demonstrated their carcinogenic properties,[5,6] a reinvestigation of the alleged relationship between boiling range and carcinogenicity of mineral oils was indicated.

## Experimental Procedures

A total of 16 fractions were available for tests in which C57 Black mice were used. The following physical and chemical data characterize the individual fractions employed which were obtained according to the scheme given in Figure 1.

*Physical and Chemical Data on Fractions.*—Fraction A-1: Shale oil distillate up to a boiling point corresponding to 380 F at 760 mm. of Hg. This material was dissolved in a mixture of olive oil (two parts) and ethanol (one part) for better solubilization,[2] producing a solution of two parts by weight of shale oil Fraction A-1 in one part of olive oil-ethanol.




*contains the bulk of the benzo[a]pyrene

Fig. 1.—Fractionation of American shale oil by thermodistillation and multiple chromatography. The N-T-U retort is a "batch" retort developed in the United States by the U. S. Bureau of Mines for the production of shale oil from Colorado oil shale. N-T-U stands for Nevada-Texas-Utah (Prien, C. H.: Oil Shale and Shale Oil, in Snell, G.: Oil Shale and Channel Coal, London, Institute of Petroleum, 1951, Vol. 2, pp. 76-111.

Fraction A-2: Shale oil distillate corresponding to a boiling range of 380-490 F at 760 mm. Fraction A-2 was dissolved in olive oil at a ratio of 2:1 by weight.

Fraction A-3: Shale oil distillate corresponding to a boiling range of 490-640 F at 760 mm. Fraction A-3 was dissolved in olive oil at a ratio of 2:1 by weight.

Fraction B-1: Shale oil distillate corresponding to a boiling range of 640-710 F at 760 mm. Fraction B-1 was dissolved in olive oil at a ratio of 2:1 by weight.

Fraction D: Residue left after distillation of shale oil to a boiling point corresponding to 950 F at 760 mm. Fraction D was mixed with olive oil at a ratio of 2:1 by weight.

All other fractions used in bioassays for carcinogenic properties were obtained by chromatographic separation of the shale oil distillate corresponding to a boiling range of 750-790 F at 760 mm.

Fraction F: Methanol eluate from first chromatographic fractionation compounds, i. e., it should correspond to the so-called "oxy" portion described by Eby[7] and obtained from catalytically cracked petroleum oils.

Fraction G-1: Cyclohexane eluate obtained from Fraction G in the second chromatographic fractionation on silica gel. From this fraction a white solid material was removed which proved to be paraffin. The remainder (Fraction G-1) retained some paraffin.

Fraction I-1: Second part of the benzene eluate obtained in the second chromatographic fractionation on silica gel.

Fraction I-2: First part of the cyclohexane-pyridine eluate obtained in the second chromatographic fractionation on silica gel.

Fraction I-2A: A middle cut of Fraction I-2 was rechromatographed. A middle cut of this chromatographic fractionation was Fraction I-2A. It weighed 0.09 gm. and was diluted for purposes of injection with 2 ml. of tri-*n*-caprylin.

Fraction I-2B: This fraction is the remainder of Fraction I-2 after removal of Fraction I-2A.

Fraction I-3: The second part of the cyclohexane-pyridine eluate and the methanolic hydrochloric acid eluate, obtained during the second chromatographic fractionation on silica gel. This fraction contains only traces of organic material.

Fraction L: Iso-octane eluate of the third chromatographic fractionation on activated alumina plus cyclohexane and cyclohexane-benzene eluate of the third chromatographic fractionation on activated alumina.

Fraction M-1: First part of the cyclohexane-benzene eluate of third chromatographic fractionation. This fraction precedes the benzo(a)pyrene fraction and, therefore, may contain traces of it.

Fraction M-3 plus M-4: Third and fourth fraction of cyclohexane-benzene eluate of third chromatographic fractionation on activated alumina. This fraction follows Fraction M-2 containing

benzo(a)pyrene and, therefore, may contain some benzo(a)pyrene.

Fraction N: Methanol eluate and pyridine eluate of third chromatographic fractionation on activated alumina. It was dissolved in equal parts by weight of olive oil.

The various fractions were tested for possible carcinogenicity by injecting 0.25 cc. to 0.3 cc. of each fraction in undiluted or diluted form, as indicated below, into the muscle tissue of the right thigh of C57 Black, Subline 6, mice, which were about 3 months old at the start of the experiment. Injections were given once a week for three successive weeks, if adequate amounts of material were available. These series of injections were repeated up to three times after treatment-free intervals of three weeks between each series for some of the fractions. The various vehicles used were not separately investigated, since their noncarcinogenicity is sufficiently established by information published by other investigators as well as in previous studies of one of us (W. C. H). Results obtained in the different series of the present experiment, moreover, serve well as control observations on the noncarcinogenicity of the vehicles.

*Scheme of Administration and Composition of Fractions.*—Fraction A-1: Two parts of A-1 diluted with one part of an olive oil-ethanol mixture (2:1); total of nine injections made in sets of three injections once weekly in three successive weeks.

Fraction A-2: Same as A-1 except that only olive oil was used as diluent; total of nine injections given.

Fraction A-3: Same as A-2; total of nine injections given.

Fraction B-1: Same as A-2; total of nine injections given.

Fraction B-2: Same as A-2; total of only four injections given because of high toxicity.

Fraction D: Same as A-2; total of six injections given because of lack of resorption of previously injected material causing undue distention of region where injection was given, with marked leakage of injected material during last injection.

Fraction L: Injected undiluted; total of six injections.

Fraction G-1: Injected undiluted; total of six injections.

Fraction F: Injected undiluted; highly toxic, therefore, administered also in two dilutions [50% and 25%] in olive oil [Fractions F-1 and F-2]; of each diluted fraction six injections were given.

Fraction I-1: Injected undiluted; one injection of 0.3 cc. only.

Fraction I-2A: Injected undiluted; one injection only of 0.2 cc.

Fraction I-2B: Injected undiluted; one injection only of 0.3 cc.

Fraction N: Diluted with equal parts of olive oil; three injections of 0.3 cc.

Fractions M-3 and M-4: Diluted with equal parts of peanut oil; two injections of 0.3 cc.

Fractions M-1: Diluted with equal parts of peanut oil; two injections of 0.3 cc.

Fraction I-3: About 1 gm. of material suspended in tri-*n*-caprylin; three injections, 0.3 cc.

The number of mice used in each series was 30, with the exception of those for which inadequate amounts of a particular fraction were available (Fractions I-1, I-2A, I-2B, M-1, M-3, and M-4). The cancerous responses obtained at the

*Period of Survival and Neoplastic Reactions on Mice Given Injections of Shale Oil Fractions*

| Fraction | Mice, No. | Months * | | | | | | | | Sarcomas, No. | Leukemias, No. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0-3 | 4-6 | 7-9 | 10-12 | 13-15 | 16-18 | 19-21 | 22-24 | | |
| | | Mice, No. | | | | | | | | | |
| A-1 | 30 | 2 | 3b | 7a | 8a | 5aa | 3 | 2 | | 4 | 1 |
| A-2 | 30 | 6 | 0 | 3 | 10 | 5a | 6 | 1 | | 1 | |
| A-3 | 30 | 2 | 1 | 5 | 12 | 6aaa | 3 | 1 | | 3 | |
| B-1 | 30 | 0 | 8 | 5 | 14aaa | 3 | | | | 3 | |
| B-2 | 30 | 10 | 1 | 10a | 5 | 2 | 1 | | | 1 | |
| D | 30 | 1 | 12 | 15 | 1 | 1 | | | | | |
| L | 30 | 1 | 6 | 7a | 10 | 8 | 4 | | | 1 | |
| G-1 | 30 | 6 | 1 | 1 | 5a | 12a | 4 | 2aa | | 4 | |
| F | 30 | 17 | 2 | 3 | 2 | 2 | 2 | 2 | | | |
| F-50% | 30 | 7 | 15 | 0 | 2 | 3 | 1 | 1 | | | |
| F-25% | 30 | 4 | 6b | 1 | 1 | 3ab | 3 | 6ab | | 2 | 3 |
| I-1 | 14 | 2 | 1 | 0 | 1 | 1 | 3 | 4 | 1 | | |
| I-2a | 10 | 0 | 1 | 1b | 0 | 1 | 1 | 4 | 2 | | 1 |
| I-2b | 15 | 0 | 0 | 0 | 0 | 3 | 1 | 5b | 6 | | 1 |
| I-3 | 30 | 8 | 2 | 0 | 0 | 1 | 6 | 7 | 9aa | 2 | |
| M-1 | 10 | 0 | 0 | 0 | 5 | 2 | 3 | | | | |
| M3-M4 | 11 | 0 | 0 | 0 | 1 | 8 | 1 | 1 | | | |
| N | 30 | 0 | 21 | 0 | 0 | 5 | 2 | 2 | | | |
| | 450 | | bb | aaa b | aaaaa | aaaaaaaa b | | aaa bb | aa | 21 | 6 |

* a indicates carcinomas involving the right thigh, i. e., the site of injection; b, leukemias and related reactions.

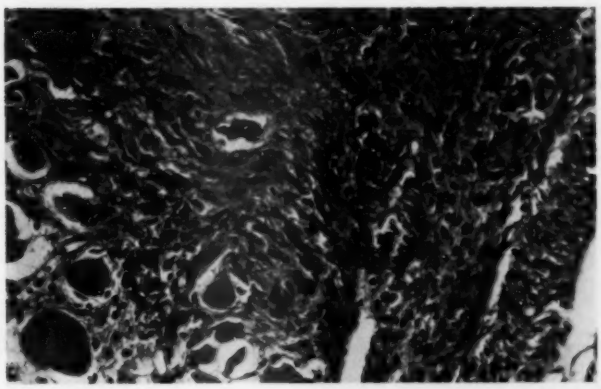


Fig. 2.—Spindle-cell sarcoma invading the skeletal muscle of the thigh; × 205.

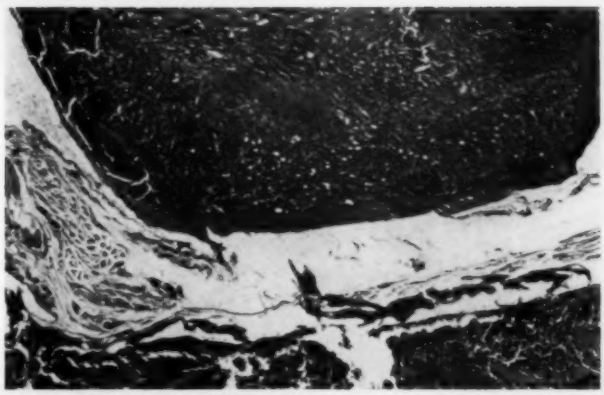


Fig. 3.—Rather well circumscribed spindle-cellular tumor located in the subcutaneous tissue of the thigh; × 87.

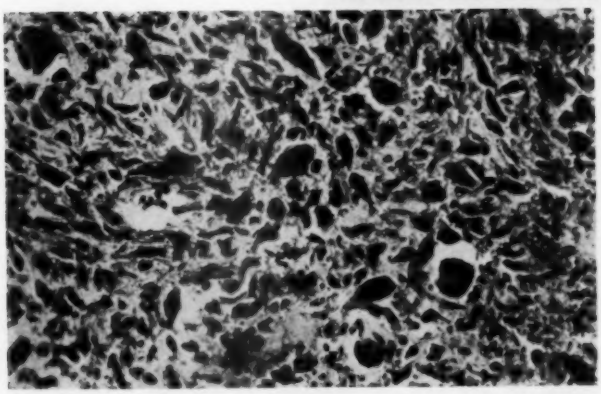


Fig. 4.—Sarcoma of the thigh, showing irregularly arranged and shaped spindle cells and giant cells; × 205.

site of injection, which were sarcomas of various types and those of a possibly systemic nature (leukemias and related conditions), as well as the time of occurrence within the experimental period are summarized in the Table.

## Results

The sarcomas involving the right thigh were white medullary firm tumors often adherent to the skin and pelvic girdle and measuring up to 5 cm. in diameter. None produced metastases despite their considerable size and their often rather anaplastic histologic structure. Direct invasion of the adjacent connective tissue, muscle, and bone, however, was common (Fig. 2). The

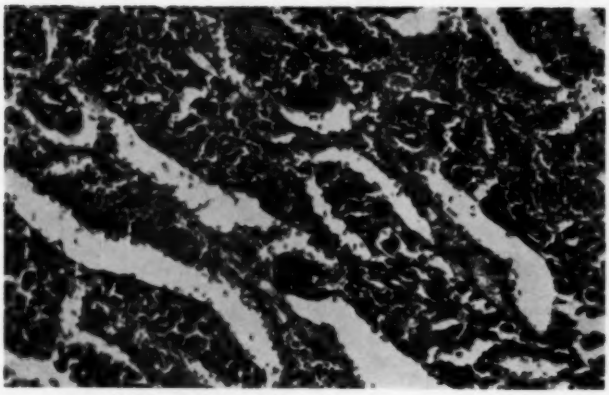


Fig. 5.—Sarcoma composed of trabecula of large round cells, forming a meshwork surrounding spaces filled with erythrocytes; × 205.

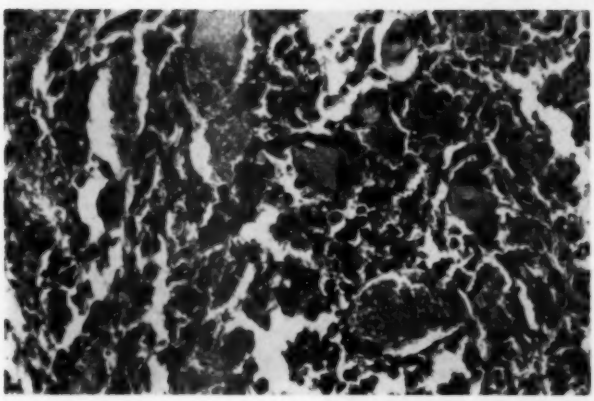


Fig. 6.—Sarcoma exhibiting in part areas of spindle cells and giant cells, in part hemangiosarcomatous structures; × 205.

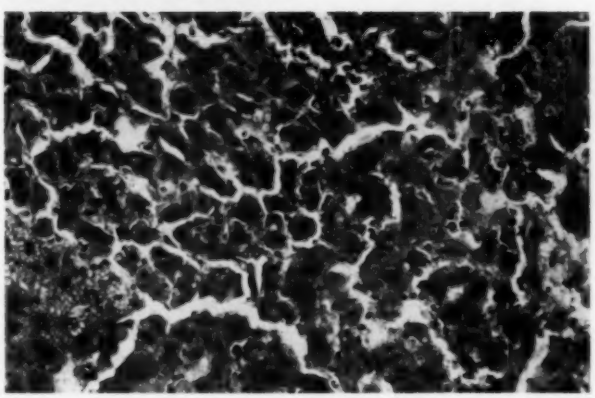


Fig. 7.—Hepatoma of the liver; × 205.

majority of the sarcomas were spindle-cellular growths (Fig. 3) some of them containing numerous and grotesque giant cells (Fig. 4). Others had areas in which a hemangiosarcomatous architecture predominated (Fig. 5), while a few exhibited hemangiosarcomatous areas in combination with accumulations of giant cells (Fig. 6). One of these tumor-bearing mice also had multiple hepatomas (Fig. 7). The hematopoietic reactions were of the leukemic and related cellular types. Their hepatic mani-

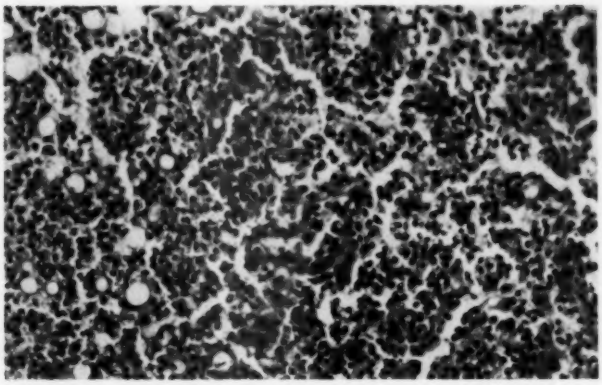


Fig. 8.—Leukemic infiltration of the liver with proliferation of Kupffer cells; × 205.

festations were sometimes associated with marked proliferations of Kupffer cells (Fig. 8).

## Comment

The observations reported indicate that shale oil fractions (Fractions A-1, A-2, A-3, B-1, B-2) boiling below the temperature range at which a benzo(a)pyrene-containing fraction is obtained and in which the presence of this chemical cannot be demonstrated by the presently available and accepted methods of analysis possess definite carcinogenic properties,[2] as evident from the development of sarcomas of the right thigh. A spontaneous occurrence of similar tumors at this site has not been observed in several thousand mice of this strain. The few lymphomatous tumors found in animals of the test series are not considered to have been induced by the oils administered but are regarded as "spontaneous" lesions.

Although the data on hand are not quite adequate for quantitative estimates of their respective carcinogenic potency, the information available nevertheless shows that the carcinogenic potency of these fractions is not inconsiderable. It is obvious from these observations that the carcinogenic power of shale oil depends only in part on its benzo(a)pyrene content and that evidently other constituents contribute to the carcinogenic effect related to crude shale oil. The experimental data obtained, moreover, reveal that the shale oil fraction (Fraction D) remaining as residue after the removal of the benzo(a)pyrene-containing fraction (Fig. 1) lacks carcinogenic power.

The specific experimental approach employed thus provides additional evidence in support of the fact that some of the distillates of shale oil, like those of synthetic hydrogenated coal oil and petroleum, boiling well below the 700 F range may possess carcinogenic properties for experimental animals. An increasing number of reports on the carcinogenic action of cutting oils of petroleum derivation on the human skin strongly suggests that the observations made in experimental animals are applicable to man.

The observations recorded also demonstrate that several subfractions of the main fraction from which the benzo(a)pyrene-containing subfraction was obtained by chromatographic separation and which are free from benzo(a)pyrene are capable of eliciting cancerous responses when intramuscularly injected (Fractions F, I-3, G-1, L) and thus play a part in determining the total cancerous reaction to this fraction. The behavior of the highly toxic Fraction F, which was tested undiluted and diluted to 50% and 25% with olive oil, is in this connection of special interest for two reasons. Eby [7] hinted at carcinogenic properties of the "oxy" portions obtained from catalytically cracked petroleum. The present findings confirm his suspicion in connection with the shale oil fraction. The results obtained

in test with this fraction indicate that a carcinogenic effect, however, becomes apparent only when the toxic action of this fraction is sufficiently depressed by diluting it to one-quarter strength (fraction−25%). Excessive toxicity of an agent which may manifest itself in necrotizing effects or in an undue shortening of the life span interferes with its carcinogenic action. The absence of carcinomatous developments from ulcerations of the skin and their rare occurrence in nasal septa of chromate manufacturers who, on the other hand, have an excessive liability to cancer of the lung seems to represent another example of the antagonistic relationship between marked toxic effects and carcinogenic action. It may also not be mere coincidence that the various carcinogenic aromatic amines (β-naphthylamine, benzidine, 4-aminodiphenyl) have a rather low toxic effect and in contrast possess a high carcinogenic potency, while their chlorinated derivatives are highly toxic and irritative to the bladder mucosa but are apparently incapable of eliciting there cancerous lesions. The ambivalent properties displayed by many carcinostatic-carcinogenic agents also provide a good illustration of this phenomenon (ionizing radiation, benzene, arsenic estrogens, mustards, urethan, etc.).

## Summary and Conclusions

Fractions obtained by thermodistillation from American shale oil and having a boiling point below that at which the benzo(a)pyrene-containing fraction is obtained possess appreciable carcinogenic properties when intramuscularly injected into C57 Black mice.

The carcinogenic potency of crude shale oil, therefore, is only in part attributable to the action of its benzo(a)pyrene content. Other chemically undertermined chemicals contribute definitely to this property of shale oil.

Some subfractions obtained by repeated chromatographic separations from the shale oil fraction containing benzo(a)pyrene also elicited sarcomas at the site of injection in mice, thereby demonstrating that the carcinogenic effect of this particular fraction also is only in part due to its benzo(a)pyrene content.

In tests on one highly toxic subfraction it was observed that cancers resulted only when its toxic effect was depressed by diluting it with olive oil to one-quarter of its original strength.

This observation provides additonal evidence supporting the view that toxic and carcinogenic properties of exogenous agents are not regularly related.

National Cancer Institute, National Institutes of Health (14).

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# Amyloidosis of the Heart

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In a study of the causes of death of 1150 persons 70 years of age and older autopsied at the Colorado General Hospital between July 1, 1940, and July 1, 1955, the 17 cases of amyloidosis of the heart observed in 16 men and 1 woman constituted 1.5% of the total. Since the ratio of men to women among these 1150 persons was 2.5:1, the corrected ratio for amyloidosis of the heart was 6.4:1. Fifteen patients were 80 to 89 years of age, and the remaining two were 72 and 77. Chronic suppuration and plasma-cell myeloma were not found in any case. The purposes of this paper are to present the pertinent available clinical details and the autopsy findings in each case, to review the literature, to describe the gross and microscopic features of amyloidosis of the heart, to compare the results of this study with those of others, and to suggest several possible reasons for the localization of amyloid in the heart, especially in the myocardium of elderly patients. The 17 cases were divided into three groups: Cases 1 through 7, in which amyloidosis of the heart caused death; Cases 8 and 9, in which cardiac amyloidosis was a cooperative cause of death, and Cases 10 through 17, in which the disease was a variably important secondary cause of death.

## Amyloidosis the Cause of Death

CASE 1.—An 89-year-old man, admitted Oct. 21, 1940, had weakness and easy fatigue for several months, nonproductive cough for two weeks; temperature, 99.2 F; blood pressure, 150/90; few moist lung rales; distinct heart sounds; soft blowing systolic murmur over mitral area; cardiac rhythm irregularly irregular; blood hemoglobin, 13.5 gm. per 100 cc.; erythrocytes, 3,750,000 per cubic millimeter; leukocytes, 14,500 per cubic millimeter; sedimentation rate, 52% in 80 minutes; hazy right apex; hilar infiltration; calcified bronchial lymph nodes; normal heart; widened sclerotic aortic arch seen by radiograph. Although he was taking sulfathiazole, he had chills and fever with temperatures to 104.6 F on Oct. 30. He died on Nov. 3, with temperature of 103.6 F and clinical diagnoses of arteriosclerotic heart disease and bronchopneumonia.

Autopsy, 11 hours after death, showed weight, 41 kg. (60% of normal [67.7 kg.]); height, 166 cm.; amyloidosis of the heart; dilatation of the heart; organized hypostatic bronchopneumonia; acute passive hyperemia of the liver, and atrophy of the liver, which weighed 945 gm. The heart weighed 300 gm., and the coronary arteries showed moderate arteriosclerosis; the brain was not examined.

*Interpretation.*—The hypostatic bronchopneumonia, which developed on the basis of left ventricular cardiac failure due to amyloidosis of the myocardium, became organized by the sulfathiazole therapy. Acute passive hyperemia of the liver indicated terminal right ventricular cardiac failure.

CASE 2.—An 81-year-old man with bilateral inguinal hernia treated by trusses for three years was admitted on Feb. 8, 1944. He had had no bowel movement for three days and had had vomitus of blood-streaked mucus for one day; faint heart sounds; nonreducible inguinal hernias; 3+ casts and 2+ erythrocytes in the urine; blood hemoglobin, 14.5 per 100 cc.; leukocytes, 12,000 per cubic millimeter; segmented neutrophils, 89%; much gas in the small intestine seen by radiograph. On Feb. 9, a loop of ileum strangulated in the right inguinal hernia sac was released. He died on Feb. 10.

Autopsy, 16½ hours after death, showed weight, 68 kg. (81% of normal [83.6 kg.]); height, 180 cm.; amyloidosis of the heart; moderate fibrosis of the myocardium; dilatation of the heart; severe edema of the lungs; left hydrothorax of 500 cc.; recent right inguinal herniorrhapy; two bands of fibrous stenosis of the ileum; corrected incarceration of the ileum; bland incarceration of the sigmoid segment of the large intestine in the left inguinal hernia sac; nodular hyperplasia of the prostate, and hypertrophy of the urinary bladder.

Submitted for publication Jan. 6, 1958.

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The heart weighed 320 gm.; the coronary arteries showed moderate arteriosclerosis; the liver weighed 1550 gm.; the brain was not examined.

*Interpretation.*—The loop of ileum intermittently incarcerated in the right inguinal hernia sac finally developed enough stenosis at two levels to interfere with its blood supply and cause symptoms of intestinal obstruction, which were adequately relieved by prompt operation. The intestinal obstruction and the operation were apparently enough to tip the myocardium damaged by abundant amyloid deposit into acute failure.

CASE 3.—An 85-year-old man, admitted from an old folks' home on Sept. 26, 1944, had dyspnea for four months, orthopnea for two months, nausea and vomiting for five days while being digitalized two months before, swelling and pain in the legs for three weeks, cough productive of yellow sputum for three weeks, dribbling, urinary frequency, and moderate diarrhea on admission; temperature, 99.2 F; pulse, 100; respirations, 80; blood pressure, 120/60; pallor; rales over lower lobe of left lung; point of maximal cardiac impulse in left second interspace; faint systolic murmur over precordium; distant heart sounds; edema of genitalia and lower extremities; 2+ leukocytes in urine; blood hemoglobin, 4.9 gm. per 100 cc.; erythrocytes 1,110,000 per cubic millimeter; leukocytes, 7700 per cubic millimeter; segmented neutrophils, 92%; erythrocytes, variable in size and shape; sedimentation, 37 mm. in one hour; hematocrit, 16.7%; greatly enlarged heart and infiltration of lower lobes of lungs seen by radiograph; and low voltage in Leads I, II, and III of electrocardiogram. He was started on a cardiac regimen and received two blood transfusions, during the second of which he died, on Sept. 28, with clinical diagnoses of arteriosclerotic heart disease and cardiac failure.

Autopsy, 20 hours after death, showed weight, 75 kg. (104% of normal [71.8 kg.]); height, 178 cm.; amyloidosis of the heart; hypertrophy of the heart, which weighed 485 gm.; dilatation of the heart; acute pericarditis; edema of the lungs; right hydrothorax of 1000 cc.; left hydrothorax of 1000 cc.; chronic passive hyperemia of liver; myeloid hyperplasia of bone marrow; nodular hyperplasia of prostate, and 9 cm. cyst of left kidney. The coronary arteries showed moderate arteriosclerosis, and the liver weighed 1275 gm.; the brain was not examined.

*Interpretation.*—This case can be properly called classic for amyloidosis of the heart in showing unmistakable signs of cardiac hypertrophy, heart failure, and low voltage in all major leads of the electrocardiograph. Determinations of blood proteins were not made, but the low hemoglobin value suggests that probably albumin was low and globulin was relatively elevated. The anatomic findings were unequivocal for amyloidosis of the heart, with lung and liver changes indicating terminal cardiac failure. Greatly increased fluid in the interstitial compartment accounted for several kilograms of body weight.

CASE 4.—An 88-year-old man, admitted Feb. 3, 1945, had dyspnea, cyanosis, and swollen ankles for 2 years; treatment with digitalis for 6 months; epistaxis for 10 days; respirations, 24; blood pressure, 120/45; pallor; edentulism; telangiectasis of mucous membrane over right ethmoid area; blood clots in nasopharynx; clear lungs; heart irregularly irregular; frequent extrasystoles; heart sounds clear at apex and distant at base; Grade 2 blowing systolic apical murmur; 2+ edema of legs; blood hemoglobin, 10 gm. per 100 cc.; erythrocytes, 3,060,000 per cubic millimeter; platelets, 120,000 per cubic millimeter; bleeding time, 15 minutes; greatly enlarged heart seen by radiograph, and residual urine, 200 cc. On Feb. 18, temperature was 102.6 F, and chest radiograph showed infiltration in middle lobe of right lung; he was given sulfadiazine, with improvement. On March 3, temperature was 102.4 F and he was given sulfathiazole. Temperature was 101.2 F to 105.4 F until death, on March 7. Clinical diagnosis was arteriosclerotic heart disease.

Autopsy, 21 hours after death, showed weight, 58 kg. (76% of normal [76.3 kg.]); height, 176 cm.; amyloidosis of the heart; hypertrophy of the heart, which weighed 530 gm.; dilatation of the heart; obliterative fibrous pericarditis; patent foramen ovale; edema of the lungs; chronic cholecystitis, and multiple (12) calculi of the gallbladder. The coronary arteries showed minimal arteriosclerosis, and the liver weighed 1165 gm.; the brain was not examined.

*Interpretation.*—The remarks for Case 3 apply for Case 4, in which unfortunately an electrocardiograph was not obtained. The decreased platelets and increased bleeding time were noteworthy. The clinical impression of hypostatic bronchopneumonia was not confirmed.

CASE 5.—A 72-year-old man, admitted on April 10, 1948, from an old folks' home, had increasing lethargy for three months, anorexia for one week; temperature, 99.8; pulse, 110; blood pressure, 90/60; coma; emaciation; pallor; skin lesions of

dorsum of hands; foul breath; very carious teeth; crackling rales in lower lobes of lungs; diminished heart sounds; increased pulmonic second sound; blood hemoglobin, 4 gm. per 100 cc.; erythrocytes, 1,500,000 per cubic millimeter; leukocytes, 6000 per cubic millimeter; lymphocytes, 72%; platelets, 100,000 per cubic millimeter; glucose, 144 to 240 mg. per 100 cc.; enlarged heart; clear lungs seen by radiograph, and inconclusive electrocardiogram. He received insulin, a blood transfusion, multivitamins, and adequate diet. On April 21, skin lesions were improved. Blood showed hemoglobin, 8 gm. per 100 cc.; erythrocytes, 1,900,000; leukocytes 18,000 per cubic millimeter; neutrophils, 74%; normoblasts, 6 per 100 erythrocytes, and platelets 100,000 per cubic millimeter. He was discharged on April 27 and readmitted May 21. Since discharge he had become disoriented, was incontinent of urine, and had developed cystitis from catheterization. He showed temperature, 101 F; pulse, 80; irregular respirations at 7; blood pressure, 104/58; lethargy; fixed pupils; ecchymoses and keratoses of skin of hands; ankle edema; 1+ albumin in urine; blood hemoglobin, 11.3 gm. per 100 cc.; erythrocytes, 3,800,000 per cubic millimeter; leukocytes, 25,000 per cubic millimeter, neutrophils, 84%; glucose, 138 mg. per 100 cc.; total proteins, 5.8 gm. per 100 cc. Despite high protein-high vitamin diet, temperature rose to 102.8 F; pulse, to 112, and respirations, to 40. He died on May 31, with clinical diagnosis of pellagra.

Autopsy, 28 hours after death, showed weight 60.4 kg. (91% of normal [66 kg.]); height, 163 cm.; amyloidosis of the heart; hypertrophy of the heart, which weighed 520 gm.; focal fibrosis of the myocardium; chronic passive hyperemia of the liver; bronchopneumonia of the lower lobe of the right lung; acute suppurative cholecystitis; acute abscesses of the kidneys; acute prostatitis; decubital ulcers of the skin of the hips and sacrum; dilatation of pancreatic acini; right obliterative fibrous pleuritis, and fibrous perisplenitis. The coronary arteries showed minimal arteriosclerosis; the liver weighed 1050 gm.; the brain and spinal cord were normal, and the heart valve measurements were not recorded.

*Interpretation.*—This case is a striking example of amyloidosis of the heart with cardiac failure accompanied by unmistakable evidence of nutritional deficiency and anemia responding somewhat to treatment. Plasma proteins were not fractionated. The electrocardiograph was not diagnostic, although the chest radiograph indicated an enlarged heart. The acute infections of the right lung, gallbladder, kidneys, prostrate, and skin were all of recent date and were not thought to contribute to cardiac amyloidosis but rather to complicate malnutrition.

CASE 6.—An 80-year-old man, admitted on Oct. 21, 1950, had dyspnea and cough productive of white mucus for two years, worsening dyspnea for two months, and ankle edema for one month; blood pressure, 100/78; moist rales in lower lobes of lungs; clear heart sounds; irregularly irregular rhythm; maximal cardiac impulse in fifth interspace 2 cm. beyond midclavicular line; accentuated pulmonic second sound; distended tense abdomen; very tender epigastrium; absent intestinal sounds; 3+ pretibial and ankle edema; blood hemoglobin, 15.5 gm. per 100 cc.; erythrocytes, 5,180,000 per cubic millimeter; leukocytes, 10,000 per cubic millimeter; neutrophils, 83%; enlarged heart; calcified aortic arch; lung infiltrations; much gas in intestines and gas between liver and diaphragm seen by radiograph; right axis deviation; atrial fibrillation at a rate of 140, and low-voltage in Lead I on electrocardiogram. On Oct. 23, he had severe epigastric pain and increasing dyspnea and cyanosis. Although Wangensteen suction was started, temperature rose to 101.8 F, and pulse, to 120; he died on Oct. 26, with clinical diagnoses of arteriosclerotic heart disease and peptic ulcer.

Autopsy, 18 hours after death, showed weight 54 kg. (75% of normal [71.8 kg.]); height, 171 cm.; amyloidosis of the heart; dilatation of the heart; edema of the lungs; perforated chronic ulcer of the duodenum with localized peritonitis; small acute ulcer of the stomach, and minimal silicosis of the lungs. The heart weighed 400 gm.; the coronary arteries showed minimal arteriosclerosis, and the liver weighed 1140 gm.; the brain was not examined.

*Interpretation.*—For reasons to be discussed, the amyloidosis of the heart and the chronic ulcer of the duodenum were thought to be due to malnutrition. Indeed, malnutrition appears to be a common denominator of cardiac amyloidosis as elaborated under Comment. The relatively high blood hemoglobin and erythrocytes, as compared to these values in some other cases presented, could have been partly due to shock with resultant hemoconcentration. The minimal silicosis of the lungs was probably not significant.

CASE 7.—An 80-year-old man, admitted March 8, 1952, had "dropsy," "heart trouble," exertional dyspnea, and orthopnea and had been treated with digitalis for five years, hospitalization for pneumonia four months before, and increasing dyspnea and swelling of the lower extremities for one month; respiration, 27; blood pressure, 140/70;

thoracic hyphosis and scoliosis; rale in lower lobes of lungs; maximal cardiac impulse in sixth interspace beyond midclavicular line; irregular intensity of heart sounds; Grade II apical systolic murmur transmitted to axilla; accentuated pulmonic second sound; liver edge 2 cm. below right costal margin; 4+ pitting edema over sacrum, legs, and feet; blood hemoglobin, 12.9 gm. per 100 cc.; erythrocytes, 4,240,000 per cubic millimeter; leukocytes, 2650 per cubic millimeter; neutrophils, 78%; hematocrit, 46%; heart, greatly enlarged, with hazy costophrenic angles seen by radiograph; left axis deviation; atrial fibrillation; many premature ventricular contractions; depressed voltage in Lead II, and inverted T wave in $V_1$ and $V_6$ on electrocardiogram. He was started on a cardiac regimen but died on March 17, with clinical diagnosis of arteriosclerotic heart disease.

Autopsy, 23½ hours after death, showed weight, 31 kg. (33% of normal [93.2 kg.]); height, 189 cm.; amyloidosis of the heart; hypertrophy of the heart, which weighed 680 gm.; dilatation of the heart; focal fibrosis of the myocardium; fibrous pericarditis; edema of the lungs; right hydrothorax of 500 cc.; left hydrothorax of 1500 cc.; early periportal cirrhosis of the liver; ascites of 1000 cc.; chronic cholecystitis; nodular goiter; absence of right testis; atrophy of left testis, and two small cysts of the left kidney. The coronary arteries showed moderate arteriosclerosis; the liver weighed 1420 gm., and amyloid was deposited in the media of the venules of the myocardium, the stroma of which was the major site of deposition in all cases; the brain was not examined.

*Interpretation.*—This patient showed unmistakable changes of malnutrition as demonstrated by the cardiac amyloidosis (a theory for the etiology of which will be discussed under Comment), severe weight loss, and early periportal cirrhosis of the liver. Diffuse myocardial damage was indicated by the electrocardiographic changes.

## Amyloidosis a Cooperative Cause of Death

Case 8.—An 89-year-old man, admitted to another hospital on Jan. 5, 1953, after collapsing on an airplane flight into Denver, was treated with quinidine, which failed to check atrial fibrillation. On Jan. 12, he was transferred to Colorado General Hospital. Examination showed blood pressure, 110/70; semicoma; restlessness; obesity; chest pain; clear lungs; left border of heart in fourth interspace 2 cm. beyond midclavicular line; cardiac rhythm very irregular; variable intensity of heart sounds; accentuated pulmonic second sound; right deep tendon reflexes increased; positive right Babinski sign; blood hemoglobin, 11.6 gm. per 100 cc.; erythrocytes, 3,130,000 per cubic millimeter; leukocytes, 6300 per cubic millimeter; atrial fibrillation; ventricular rate, 130; notched QRS in Leads II, III, $aV_F$, $V_5$; increased depth of S in Leads $V_{1-4}$; reduced voltage in Leads I, II, and III, on electrocardiogram. The patient died on Jan. 14, with clinical diagnosis of acute infarction of the left ventricle of the heart with left bundle-branch block.

Autopsy, 19 hours after death, showed weight 70 kg. (about normal [69.5 kg.]); height, 167 cm.; arteriosclerosis of the coronary arteries; stenosis of the anterior descending branch of the left coronary artery; large recent infarct of the left ventricle of the heart; mural thrombosis of the left ventricle of the heart; hypertrophy of the heart, which weighed 570 gm.; amyloidosis of the heart; dilatation of the heart; edema of the lungs; right hydrothorax of 1000 cc.; left hydrothorax of 1000 cc.; minimal nodular hyperplasia of the prostate, and diverticula of the sigmoid segment of the large intestine. The liver weighed 1300 gm., and the brain was normal.

*Interpretation.*—In the absence of sufficient coronary arteriosclerosis to cause stenosis of a major branch of the left coronary artery and fatal myocardial infarction, the patient would probably have eventually died of the severe amyloidosis of the myocardium, which contributed significantly to death. The anatomic evidences of cardiac failure were unequivocal.

Case 9.—An 88-year-old man, admitted Sept. 22, 1953, had attacks of syncope for two years; blood pressure, 104/58; drowsiness; edentulism; rales in lower lobes of lungs; point of maximal cardiac impulse in left fifth interspace just lateral to midclavicular line; faint heart sounds; soft Grade 2 apical systolic murmur; liver, 2 cm. below right costal margin; 2+ edema of legs and feet; 2+ albumin in urine; blood hemoglobin, 9.5 gm. per 100 cc.; erythrocytes, 3,400,000 per cubic millimeter; leukocytes, 9000 per cubic millimeter; neutrophils, 75%; hematocrit, 29%; prothrombin time, 16 seconds or 52%; infiltration in lower lobe of left lung; osteoporosis of visualized bones seen by radiograph; left axis deviation; low voltage in Lead II, T wave inverted in Leads II, III, $aV_F$, $V_{1-6}$, seen by electrocardiograph. Digitalis abated the cardiac symptoms. He was discharged on Oct. 1. He was readmitted on Dec. 3. Examination showed temperature, 100.6 F; blood pressure, 120/40; semicoma; dehydration; incontinence of urine; lungs clear; same heart findings; liver not palpable; hypoactive reflexes; 3+ albumin in urine; essentially same blood cell count; negative

chest radiograph; left axis deviation, atrial fibrillation, and high-grade atrioventricular block found on electrocardiogram. He died on Dec. 12, with clinical diagnosis of arteriosclerotic heart disease in congestive failure.

Autopsy, 21 hours after death, showed weight, 48 kg. (71% of normal [67.7 kg.]); arteriosclerosis of the coronary arteries; small recent infarct of the right ventricle of the heart; amyloidosis of the heart; edema of the lungs; right hydrothorax of 400 cc.; left hydrothorax of 500 cc.; arteriosclerotic stenosis of the superior mesenteric artery; early gangrene of the ileum; acute fibrinous peritonitis; arteriosclerosis of the kidneys; early bronchopneumonia; chronic cystitis, and fibrous perisplenitis. The heart weighed 375 gm., and the liver weighed 1040 gm.; the brain was not examined.

*Interpretation.*—The lesions of recent infarction of the myocardium of the right ventricle and cardiac amyloidosis were related to clear-cut signs of cardiac failure. Weight loss and anemia indicated malnutrition. The localization of the small infarct of the right ventricle of the heart was not stated in the protocol, but involvement of the atrioventricular node by the infarct or by amyloid deposit or both could account for the atrioventricular block observed in the electrocardiograph. Early gangrene of the ileum due to arteriosclerotic stenosis of the mesenteric artery was a terminal event.

## Amyloidosis a Secondary Cause of Death

CASE 10.—An 84-year-old man, admitted July 27, 1942, had epigastric pain not relieved by food for six months and anorexia and nausea for three months; no recorded blood pressure; few carious teeth remaining; clear lungs; distant heart sounds; epigastric mass; 3+ albumin in urine; blood erythrocytes, 4,900,000 per cubic millimeter; leukocytes, 4,700 per cubic millimeter; neutrophils, 72%; severe cardiospasm; large filling defect in antrum of stomach and first part of duodenum; some six-hour gastric residue seen by radiograph; incomplete bundle-branch block and borderline atrioventricular block on electrocardiogram. The patient died on Aug. 2, with clinical diagnoses of carcinoma of the stomach, coronary arteriosclerosis, and myocardial infarction.

Autopsy, 2½ hours after death, showed weight 52.2 kg. (81% of normal [64.5 kg.]); height, 158 cm.; mucous-cell carcinoma of the stomach with metastases to regional and periaortic lymph nodes; ascites of 500 cc.; fibrous peritonitis; right hydrothorax of 700 cc.; left hydrothorax of 700 cc., and amyloidosis of the heart. The heart weighed 290 gm.; the coronary arteries showed minimal arteriosclerosis; the liver weighed 1110 gm., and many corpora amylacea filled the alveoli of the lungs; the brain was not examined.

*Interpretation.*—The carcinoma of the stomach was thought to be the major cause of death, with involvement of lymphatics by metastases resulting in ascites and hydrothorax. Probably the amyloidosis of the heart was partly responsible for recurrent edema of the lungs indicated by the corpora amylacea in the pulmonary alveoli as well as for the bilateral hydrothorax.

CASE 11.—An 87-year-old man, admitted April 1, 1949, had an episode of chills, fever, and left thoracic pain 16 months before; three to eight stools daily and 30 lb. weight loss for 2 months; watery stool and alternating constipation and diarrhea for 10 days; anorexia, dysphagia, and epigastric pain with solid food; dysuria, dribbling, and pain in urinary bladder; blood pressure, 106/66; brittle nails; edentulism; red fissured tongue; easily stimulated carotid sinus reflex; painful 7 cm. lump over left seventh rib; clear lungs; faint heart sounds; Grade 2 systolic murmur over aortic area into neck; 8 cm. epigastric mass; right indirect inguinal hernia; retention catheter in place; 4+ enlargement of prostate; 1+ albumin and 3+ leukocytes in urine; blood hemoglobin, 8.5 gm. per 100 cc.; erythrocytes, 2,810,000 per cubic millimeter; heart and lungs clear, and soft tissue mass along left seventh rib seen by radiograph. He was started on a cardiac regimen and received two blood transfusions. On April 15, gastrointestinal radiographs showed obstruction of distal third of aorta and loop of small intestine in right inguinal hernia sac but were otherwise negative. Barium enema was negative. On May 7, biopsy of the left thoracic mass revealed an unclassified cancer. Between May 20 and June 6, a total of 3800 r was delivered to this mass. The patient died on June 7.

Autopsy, 10½ hours after death, showed weight 43 kg. (60% of normal [71.8 kg.]); height, 170 cm.; undifferentiated carcinoma of the jejunum with metastases to the left thoracic region, iliac and bronchial lymph nodes, and right lung; atrophy of the liver, which weighed 960 gm.; x-irradiation hypoplasia of bone marrow; amyloidosis of the heart, pancreas, adrenals, and seminal vesicles; dilatation of the heart; edema of the lungs; right hydrothorax of 150 cc.; left hydrothorax of 500 cc.; acute passive hyperemia of the liver; nodular hyperplasia of the prostate six weeks after perineal resection; partial absence of the prostate; perineal scar; chronic cystitis, and acute pyelonephritis.

The heart weighed 290 gm., and the coronary arteries showed minimal arteriosclerosis; the brain was not examined. Amyloid was deposited chiefly in the media of the blood vessels of the heart, adrenals, pancreas, and seminal vesicles; in the endocardium, and relatively little in the myocardium.

*Interpretation.*—The carcinoma of the jejunum was the chief cause of death and promoted malnutrition, which in turn contributed to the amyloidosis of the heart and blood vessels in other organs. Cardiac failure was indicated by dilatation of the heart, edema of the lungs, hydrothorax, and acute passive hyperemia of the liver. The chronic cystitis and acute pyelonephritis were not thought to contribute to the amyloidosis.

CASE 12.—An 80-year-old man, admitted Sept. 14, 1951, in semicoma without history, had respirations, 40; blood pressure, 80/55; carious teeth; rales throughout the lungs; left border of heart at midclavicular line; negative neurologic findings; blood hemoglobin, 13 gm. per 100 cc.; erythrocytes, 4,500,000 per cubic millimeter; leukocytes, 14,200 per cubic millimeter; neutrophils, 82% (stab forms, 77%); nonprotein nitrogen, 98 mg. per 100 cc.; carbon dioxide combining power, 39 vol.%; infiltrations in the lower lobes of the lungs and cloudy costophrenic angles seen by radiograph. On Sept. 15, penicillin was started and a Foley catheter was inserted. The patient died on Sept. 16.

Autopsy, 31 hours after death, showed weight, 60 kg. (89% of normal [67.7 kg.]); height, 164 cm.; severe bilateral bronchopneumonia; amyloidosis of the heart; hypertrophy of the heart, which weighed 440 gm.; edema of the lungs; left hydrothorax of 200 cc.; arteriosclerosis of the aorta; mural thrombosis of the aorta, and left hydrocele. The intermuscular deposit of amyloid in the heart was relatively little as compared to that in most of the cases studied; the coronary arteries showed moderate arteriosclerosis, the liver weighed 1550 gm., and the brain was normal.

*Interpretation.*—The chief cause of death was overwhelming bronchopneumonia with circulatory collapse, in small measure due to cardiac amyloidosis. The clinical evidences of severe acute infection included the auscultatory findings in the lungs, the findings on chest radiograph, the moderate leukocytosis, and the shift to the left of the blood neutrophils.

CASE 13.—An 85-year-old man had diabetes mellitus variably controlled by diet and insulin for nine years, an admission in May, 1949, for ulcerated lesions on the toes, an admission in September, 1950, for skin rash, and reddening and swelling of the right foot for one month before final admission, on Feb. 6, 1951. Examination showed blood pressure, 140/70; edentulism; clear lungs; low-pitched systolic murmur in left third interspace into apex and neck; 2+ edema of distal half of right leg and of right foot; dry second right toe; absent right posterior tibial and dorsalis pedis pulses; 1+ albumin in urine; blood hemoglobin, 12 gm. per 100 cc.; erythrocytes, 4,920,000 per cubic millimeter; leukocytes, 14,700 per cubic millimeter; neutrophils, 64%; glucose, 108 to 246 mg. per 100 cc.; total protein, 6.5 gm. per 100 cc. He received diabetic diet, insulin, anticoagulants, and soaks to right leg and foot. On March 9, electrocardiogram showed an asymptomatic myocardial infarct, confirmed by three other readings in the next month. On April 10, the right lower extremity was amputated through the middle of the thigh. Recovery was smooth. Diabetes mellitus was well controlled. On May 5, the patient was discharged to a convalescent home, where he died on Sept. 18.

Autopsy, about 12 hours after death, showed weight, 75 kg. (90% of normal [83.6 kg.] and accounted for by absence of the right lower extremity); height, 189 cm.; diabetes mellitus, clinical; arteriosclerosis of the coronary arteries; occlusion of the right coronary artery; small old infarct of the left ventricle of the heart; amyloidosis of the heart, hypertrophy of the heart, which weighed 500 gm.; edema of the lungs; right hydrothorax of 1500 cc.; left hydrothorax of 1500 cc.; acute passive hyperemia of the liver; arteriosclerosis of the arteries of the right lower extremity (five months postoperative); thrombosis of the right tibial arteries; gangrene of the right foot; midthigh absence of the right lower extremity; occult adenocarcinoma of the prostate; chronic cystitis; chronic cholecystitis; calculi (three), gallbladder; calculus, common bile duct, and fibrous perisplenitis. The liver weighed 1660 gm.; the brain was not examined.

*Interpretation.*—This patient showed two manifestations of diabetes. The first was the severe arterosclerosis of the arteries of the right lower extremity, successfully treated by midthigh amputation. The second was severe arteriosclerosis of the coronary arteries, resulting in a small infarct of the left ventricle of the heart, which, coupled with the amyloidosis of the heart in producing cardiac failure, was the immediate cause of death.

CASE 14.—An 87-year-old man, admitted June 14, 1952, had three attacks of pneumonia in the

preceding six years while in a convalescent home; increasing weakness, anorexia, and lethargy for one month; temperature, 101; pulse, 108; shallow respirations, 26; blood pressure, 120/64; coma; general muscle rigidity; moderate rales in the lungs; faint heart tones; 2+ enlarged prostate; 1+ albumin in urine; blood hemoglobin, 11.5 gm. per 100 cc.; erythrocytes, 4,600,000 per cubic millimeter; albumin, 2.02 gm. per 100 cc.; globulin, 2.35 gm. per 100 cc. On June 17, he developed left hemiparesis. On July 20, he had melena. On July 22, temperature was 100.4 F; pulse, 100; respirations, 28; blood contained 19,200 leukocytes per cubic millimeter, with 97% neutrophils; lower lobes of lungs contained rales, and radiograph showed infiltration of the lower lobe of the right lung. Electrocardiograph revealed sinus bradycardia, first-degree heart block, and left bundle-branch block. He received penicillin and tracheal suction but failed rapidly. Just before death, on July 24, electrocardiogram showed complete atrioventricular dissociation.

Autopsy, 12 hours after death, showed weight, 58 kg. (83% of normal [70 kg.]); height, 167 cm.; bronchopneumonia of lower lobes of lungs; arteriosclerosis of cerebral arteries; stenosis of right middle cerebral artery; infarct of right parietal lobe of the brain; nodular hyperplasia of the prostate; hypertrophy of the urinary bladder; severe chronic cystitis; acute ulcerative prostatitis; acute ulcer of the large intestine; mural thrombosis of the mitral valve of the heart; amyloidosis of the heart; hypoplasia of the bone marrow; atrophy of the liver, which weighed 1000 gm.; 4 cm. cyst of the right kidney, and fibrous perisplenitis. The amyloid in the myocardium was relatively little as compared to that in most of the cases studied; the heart weighed 270 gm., and the coronary arteries showed minimal arteriosclerosis.

*Interpretation.*—The major cause of death was bronchopneumonia. The left hemiparesis was explained by the infarct of the right parietal lobe of the brain due to the arteriosclerotic stenosis of the right middle cerebral artery. The acute ulcers of the large intestine could have been related to the penicillin therapy, but culture to confirm an organism, such as a hemolytic Staphylococcus, was not made. The amyloidosis of the heart was relatively little, but a small amount of amyloid strategically placed could have interfered with conduction through the atrioventricular node and beyond, a possibility indicated by the electrocardiographic findings. Fractionation of blood albumin and globulin was made only in this patient and in Cases 16 and 17. In Cases 14 and 17, the relative increase of globulin could have contributed to the deposit of amyloid in the myocardium.

CASE 15.—An 81-year-old man, observed in the outpatient department in December, 1950, for arteriosclerotic heart disease treated by glyceryl trinitrate (nitroglycerin), was admitted on May 27, 1953, with exertional dyspnea, orthopnea, ankle edema, and palpitation for two years; weight loss for one year; anorexia, nausea, and epigastric pain, worse with eating for four months; increasing ankle edema for two months; tarry stool for one month, and vomiting of black fluid for three weeks. Examination showed blood pressure, 102/60; pallor; brown furrowed tongue; clear lungs; distinct heart sounds; Grade 2 basal systolic murmur; liver edge 2 cm. below right costal margin; enlarged prostate; tarry stool; 2+ edema of legs and feet; blood hemoglobin, 3.5 gm. per 100 cc.; erythrocytes, 1,750,000 per cubic millimeter; leukocytes, 21,700 per cubic millimeter; neutrophils, 90%; hematocrit, 13%; total protein, 4.4 gm. per 100 cc.; stool, 4+-positive for occult blood; obstruction of middle of transverse segment of large intestine by barium enema; left axis deviation; left ventricular strain, and tall R in $V_4$ and $V_5$ on electrocardiogram. He received eight blood transfusions. On June 6, he developed symptoms of obstruction of the large intestine. Mikulicz colostomy of the transverse segment of the large intestine was done. On June 8, blood hemoglobin was 6.5 gm. per 100 cc.; erythrocytes, 2,300,000 per cubic millimeter; leukocytes, 15,900 per cubic millimeter; neutrophils, 91%; hematocrit, 25%; nonprotein nitrogen, 115 mg. per 100 cc., and carbon dioxide combining power 30 vol.%. The patient died on June 10.

Autopsy, 17 hours after death, showed weight 56 kg. (78% of normal [71.8 kg.]); height, 170 cm.; two chronic ulcers of the duodenum; massive hemorrhage in stomach and intestines; recent colostomy of the transverse segment of the large intestine; amyloidosis of the heart; edema of the lungs; right hydrothorax of 200 cc.; left hydrothorax of 500 cc.; acute passive hyperemia of the liver; 2 cm. infarct of the spleen; bronchopneumonia; minimal nodular hyperplasia of the prostate; infarct of the prostate, and diverticula of the sigmoid segment of the large intestine. No cause for obstruction of the large intestine was demonstrated. The heart weighed 360 gm.; the coronary arteries showed minimal arteriosclerosis; the liver weighed 1450 gm., and the brain was normal.

*Interpretation.*—As in Case 6, the amyloidosis of the heart and the chronic ulcers of the duodenum were thought to be due

to malnutrition. In Case 15, the chronic ulcers were responsible for the fatal massive gastrointestinal bleeding. The amyloidosis of the heart accompanied by cardiac failure contributed to death. The low total blood proteins indicated possible low albumin and relatively high globulin values. The very low blood hemoglobin mainly due to severe gastrointestinal bleeding contributed to the depression of the blood proteins.

Case 16.—An 83-year-old man, admitted Aug. 26, 1954, had gradually worsening dysphagia and vomiting of solid food for one year, interpreted elsewhere as due to carcinoma of the esophagus. Examination showed temperature, 99 F; blood pressure, 90/40; clear lungs; point of maximal cardiac impulse in left fifth interspace 9 cm. to left of midsternal line; Grade 3 precordial systolic murmur; blood hemoglobin, 15 gm. per 100 cc.; erythrocytes, 5,210,000 per cubic millimeter; albumin, 3.65 gm. per 100 cc.; globulin, 2.27 gm. per 100 cc.; lesion at distal end of esophagus interpreted as carcinoma and small paraesophageal sliding hiatus hernia seen by radiograph; T wave inverted in Leads I and $V_{4-6}$ on electrocardiogram. On Aug. 30, esophagoscopy showed a rigid constriction 4 cm. above the diaphragm and prohibiting passage of the esophagoscope. On Sept. 8, a feeding jejunostomy was made. The patient failed rapidly, vomited, and aspirated vomitus. Temperature was 100 F and pulse 102 when he died, on Sept. 12.

Autopsy, 23½ hours after death, showed weight 46 kg. (71% of normal [64.5 kg.]); height, 160 cm.; stricture of the esophagus; recent jejunostomy; acute jejunitis; recent laparotomy wound; recent tracheostomy; bronchopneumonia, and amyloidosis of the heart. The heart weighed 290 gm.; the coronary arteries showed moderate arteriosclerosis, and the liver weighed 1150 gm.; the brain was not examined.

*Interpretation.*—The dysphagia related to the fibrous stricture of the esophagus was contributory to weight loss. The relatively high blood hemoglobin and erythrocytes were surprising in view of his poor general condition and may have been due to hemoconcentration resulting from vomiting and restricted fluid intake. The actual cause of the fibrosis of the esophagus at the site of stricture was not ascertained. The immediate cause of death was bronchopneumonia. The amyloidosis of the heart was well developed but did not contribute significantly to death.

Case 17.—A 77-year-old woman, admitted on May 17, 1955, had a right upper quadrant mass and weight loss of 9 kg. for three months; cough, dyspnea, anorexia, and incontinence of urine for one week; right upper quadrant abdominal pain for three days; temperature, 100 F; pulse, 104; respirations, 24; blood pressure, 134/68; clear lungs; Grade 2 systolic murmur in left fourth interspace; 10 cm. rounded mass in the right upper quadrant of the abdomen; liver edge 5 cm. below the right costal margin; hard feces in the descending segment of the large intestine; feces, 4+ guaiac-positive; blood hemoglobin, 8.5 gm. per 100 cc.; hematocrit, 26%; leukocytes, 13,500 per cubic millimeter; neutrophils, 92%, prothrombin time, 16 seconds or 59%; albumin, 2.14 gm. per 100 cc.; globulin, 3.55 gm. per 100 cc.; osteoporosis of visualized bones and enlarged heart seen by radiograph; left axis deviation and small Q in Leads I, $aV_L$, $V_5$, and $V_6$ on electrocardiogram. Gastrointestinal series showed barium passing through the stomach into a 15×10 cm. cavity enclosing gas and a little barium within the jejunum, indicating carcinoma of the gallbladder invaded into the duodenum. The patient received two blood transfusions and died on June 6.

Autopsy, 13 hours after death, showed weight, 43 kg. (70% of normal [61.4 kg.]); height, 154 cm.; undifferentiated carcinoma of the gallbladder extended into liver, duodenum, and large intestine; cholecystoduodenal fistula; amyloidosis of the heart; edema of the lungs; 17 mm. cortical adenoma of the left adrenal; old laparotomy scar; absence of the appendix, and ventral hernia. The heart weighed 380 gm.; the coronary arteries showed minimal arteriosclerosis, and the liver weighed 1800 gm.; the brain was not examined.

*Interpretation.*—The bulky carcinoma of the gallbladder was adequate explanation for the malnutrition of this patient, indicated by the severe anemia and the low level of blood albumin. The possibility that the relatively increased blood globulin was a factor in causing amyloidosis of the heart is to be discussed under Comment. The liver weight included the invaded carcinoma. Actual liver parenchyma was relatively little and more in keeping with the smaller or atrophic livers in most of the other cases. The abundant amyloid in the myocardium contributed to cardiac failure, denoted by the edema of the lungs.

## Comment

In assessment of the cases of amyloidosis of the heart in the literature, these have been

divided into two groups: (1) those in which amyloidosis was localized principally to the heart and (2) those in which amyloidosis affected the heart and other sites of variable significance. The emphasis in the discussion will be placed upon the first group.

The incidence of amyloidosis of the heart was 3.47% in patients past 50 years of age (Jones and Frazier, 1950[30]), 13% in patients over 70 years of age (Hüsselmann, 1955[27]), and 1.5% in patients 70 years of age and older (the present series).

Amyloidosis localized principally to the heart was the cause of death in 21 cases and a contributory cause of death in 23 cases described in the following papers: Beneke and Bönning (1908),[4] Kann (1922),[33] Larsen (1930),[41] Budd (1934),[7] Ranström (1946),[52] King (1948),[35] Ballinger (1949),[1] Dahlin (1949),[11] Hulbert and Meyer (1949),[26] Holzmann (1950),[24] Jones and Frazier (1950),[30] Josselson et al. (1951),[32] Ranström (1951),[53] Smith (1951),[60] Cornelius (1952),[10] Thomashow et al. (1953),[64] Jackson and Slavin (1954),[29] Loogen and Böhm (1954),[45] and Lee and Kaufmann (1957).[42] In the present series, cardiac amyloidosis caused death in seven patients, cooperated in causing death in two, and contributed variably to death in eight.

These 44 cases of amyloidosis localized principally to the heart included 32 men and 12 women, or a ratio of 2.66:1, compared to that of 1.64 of Josselson (1952)[31] for his 29 patients, to that of 1.75 of Hüsselmann (1955)[27] for his 40 patients, and to that of 6.4 for the 17 patients in the present series.

These 44 cases showed the following age distribution: 30 to 39 years, two; 40 to 49, one; 50 to 59, four; 60 to 69, four; 70 to 79, fifteen; 80 to 89, fifteen, and 90 to 99, three. Thus 75% were 70 years of age and older. Jones and Frazier (1950)[30] observed this age preponderance in 70% of their cases; Josselson (1952),[31] in 90%, and Hüsselmann (1955),[27] in 92%. The 100% incidence in the present series is not corrected by possible cases occurring before the age of 70 years, since the autopsies of patients under this age at the Colorado General Hospital were not analyzed for cardiac amyloidosis.

Among 54 patients with amyloidosis of the heart and other sites of variable significance, 28 were men and 26 were women, or a ratio of 1.1:1. Only five, or 9.3%, were 70 years of age and older. These patients had symptoms, signs, and anatomic findings related to (1) systems other than the cardiovascular system, (2) the cardiovascular system and one or more other systems, or (3) the cardiovascular system only. These 54 cases were reported by the following authors: Wild (1886),[70] Steinhaus (1902),[62] Silver and Lindblom (1926),[58] Lubarsch (1929),[46] Warren (1930),[68] Koller (1932),[39] Bannick et al. (1933),[2] Perla and Gross (1935),[51] Reimann et al. (1935),[54] Ferris (1936),[16] Kerwin (1936),[34] Barnard et al. (1938),[3] Bürümcekci (1938),[8] Koletsky and Stecher (1939),[38] Binford (1940),[5] Pearson et al. (1941),[50] Dillon and Evans (1942),[14] Sappington et al. (1942),[56] Soisalo and Ritama (1943-1944),[61] Golden (1945),[20] Lindsay and Knorp (1945),[44] Eisen (1946),[15] Lindsay (1946),[43] Findley and Adams (1948),[17] Iverson and Morrison (1948),[28] Wessler and Freedberg (1948),[69] Ballinger (1949),[1] Dahlin (1949),[11] Hartney et al. (1949),[22] DeWolf and Clarke (1950),[13] Parker et al. (1950),[49] Wahi (1950),[67] Woolf (1950),[73] Fisher and Preuss (1951),[18] Ranström (1951),[53] Wiley et al. (1951),[71] Williams (1952),[72] Cohen (1953),[9] Hirsch (1953),[23] Strich and Wade (1953),[63] Tribedi and Roy (1953),[65] Ohlinger and Harmos (1954),[48] and van der Straeten et al. (1954).[66] Other papers, not read because of language, were those of Ritama and Saksela (1949),[55] Seip (1950),[57] Hruska (1954),[25] Küley et al. (1954),[40] and Frederiksen (1955).[19]

The foregoing analysis indicates that amyloidosis of the so-called primary type is more widespread in body organs and tissues before the age of 70 years and

tends to be localized to the heart and to be commoner in men past this age.

The symptoms of patients with cardiac amyloidosis included dyspnea, orthopnea, cyanosis, and cough. Symptoms of cardiac failure were the only features or coexisted with or were masked by symptoms related to involvement of other organ systems by other diseases which were the major causes of death. The signs in patients with cardiac amyloidosis included low blood pressure, peripheral edema, anasarca, rales in the lungs, normal or hypertrophied heart, systolic murmur, distant or muffled heart tones, accentuated pulmonic second sound, and atrial fibrillation. Ballinger (1949)[1] emphasized that the cardiac failure observed in amyloidosis does not respond to treatment as readily as that in arteriosclerotic heart disease or essential hypertension.

Fifteen of the patients in the current series showed 33% to 91% of normal weight based on the height-weight figures of McLester and Derby (1952).[47] In the two patients of normal weight, abnormal accumulation of fluid in body cavities and tissues accounted for some body weight. In Josselson's series (1952),[31] many of the patients were 4.5 kg. or more underweight.

Other evidences of malnutrition in the present series included teeth absent or few in all 14 patients in whom examination of the teeth was recorded, a blood hemoglobin value of 13 gm. per 100 cc. or below, and erythrocytes, 4,000,000 per cubic millimeter or below in 12 patients and a liver weight of 1200 gm. or less in 9 patients. Case 2 of Ranström (1945)[52] and the cases of Josselson et al. (1951)[32] and of Jackson and Slavin (1954)[29] showed notable anemia.

Otherwise, data in the literature for body weight, condition of teeth, blood hemoglobin and erythrocytes, and liver weight were not available for analysis.

In the literature and in the present series, the chest radiograph showed a normal or hypertrophied heart, infiltrations in the lungs indicative of pulmonary edema, and cloudy costophrenic angles denoting hydrothorax.

The electrocardiographic findings recorded in the literature included atrial fibrillation, left ventricular strain, left axis deviation, inverted or flat T wave, increased P-R interval, notched QRS, atrioventricular block, bundle-branch block, prolonged atrioventricular conduction time, and premature ventricular contractions (Larsen, 1930[41]; Ballinger, 1949[1]; Dahlin, 1949[11]; Hulbert and Meyer, 1949[26]; Holzmann, 1950[24]; Jones and Frazier, 1950[30]; Josselson, 1952,[31] and Thomashow et al. 1953[64]). Loogen and Böhm (1954)[45] summarized the principal changes as follows: low voltage of ventricular complex, low amplitude of atrial complex, delayed intraventricular conduction, partial atrioventricular block, and alterations suggesting myocardial ventricular damage or infarct. The observations recorded for Cases 3, 6 through 10, and 13 through 17 of the present series agree substantially with those summarized from the literature.

The gross findings in the heart were adequately described by Beneke and Bönning (1908),[4] who noted that the endocardium of the atria was studded with minute translucent nodules also seen in the myocardium, especially in the ventricles. Dahlin and Edwards (1949)[12] emphasized the granules in the endocardium of the atria and their absence from the endocardium of the ventricles. They also observed granules in the epicardium and in the atrioventricular valves. Jones and Frazier (1950)[30] described red-brown myocardium with a pale gray cast and the accentuation of gray amyloid among muscle bundles by formaldehyde fixation. They found amyloid granules most striking in the endocardium of the right atrium, especially over the interatrial septum, near the coronary venous sinus, and over the superior surface of the tricuspid valve. In the most severely involved hearts, the amyloid contributed 75% to 95% of the bulk of the wall of the chambers, of which the left ventricle showed the greatest deposit. No paper consulted described the distribution of amyloid in relation to the conduction system of the heart,

an important type of investigation in the elucidation of some electrocardiographic changes. In the present series, amyloid deposits in the heart were not recognized grossly in any instance. In the literature, the recorded weight of the heart was 300 to 700 gm. In the present series, the heart weight was 290 to 680 gm.

In 12 cases in the literature in which coronary arteries were described (Beneke and Bönning, 1908[4]; Ranström, 1946[52]; Ballinger, 1949[1]; Hulbert and Meyer, 1949[26]; Holzmann, 1950[24]; Cornelius, 1952[10]; Thomashow et al. 1953[64]; Jackson and Slavin, 1954[29]), coronary arteriosclerosis was minimal or moderate in 8 and severe in 4. In the present series, coronary arteriosclerosis was minimal to moderate and nonocclusive in 14 and severe and occlusive in 3.

Microscopic findings for cardiac amyloidosis were variably complete in all papers consulted. Because of the numerous excellent photomicrographs available in such contributions as those of Larsen (1930),[41] Ranström (1946),[52] King (1948),[35] Jones and Frazier (1950),[30] Josselson (1952),[31] and Lee and Kaufmann (1957),[42] photomicrographs have not been included in the present communication. Beneke and Bönning (1908)[4] observed that the stroma of the myocardium of all chambers was heavily infiltrated with amyloid, also deposited in the media of venules and in the atrial endocardium. Kann (1922)[33] and Larsen (1930)[41] found the stroma of all layers of the heart involved and the blood vessels, especially the veins. Larsen was insistent that amyloid did not occur in myocardial arteries. Ranström (1946)[52] described the methyl violet metachromasia of the amyloid in the stroma and blood vessels of the myocardium. King (1948)[35] confirmed his observations, noted that the amyloid was positive with Congo red, and commented that the substance stained in a tone different from the shades for collagen and reticulum when an ammoniacal silver stain devised by Del Rio-Hortega was applied to fresh frozen sections. Dahlin and Edwards (1949)[12] studied reticulum stains counterstained by methyl violet. The amyloid was deposited among muscle bundles and capillaries. The deposit was patchy, with the largest accumulation 1 mm. in diameter or less frequently diffuse. The muscle fibers were ringed by amyloid, were shrunken, or had disappeared, depending on the abundance of the material metachromatic with methyl violet and deep red with Congo red. Jones and Frazier (1950)[30] were impressed in the reticulum stain by the intimate association of amyloid with the reticulum of the heart. In severe involvement, they found that the reticulum became thicker, irregular, fragmented, or destroyed as groups of muscle fibers were progressively invested, reduced in size, and ablated by amyloid, which they believed might be derived from the degenerated reticulum. Josselson (1952)[31] quantitated the amount of cardiac amyloid with age in his 29 cases, in which the amount was mild in 8 patients 63 to 89 years of age, moderate in 8 patients 75 to 90, and severe in 13 patients 75 to 100. In the present series, the amyloid was deposited chiefly in the stroma of the myocardium and was most abundant in the ventricles. The amyloid was plentiful in the myocardium of all but Cases 11, 12, and 14, in which involvement was relatively little. In Case 7, the media of the myocardial venules was also affected. The amyloid was confined to the heart in all instances but Case 11, in which the media of the blood vessels of the heart, adrenals, pancreas, and seminal vesicles and the endocardium was also infiltrated. The features observed by Dahlin and Edwards (1949)[12] and Jones and Frazier (1950)[30] for the hematoxylin and eosin appearance of amyloid in the myocardial stroma were confirmed in the present series, in which this substance was metachromatic with crystal violet, pale orange to deep orange-red with Congo red, moderately blue with the periodic acid-Schiff (PAS) routine, dull green with the Gomori trichrome stain, an indifferent golden shade

in the Wilder reticulum stain, and very pale purple with the Weigert elastic-tissue stain counterstained by the Van Gieson method. The PAS stain was interpreted as negative. The reticulum fibers were variably displaced but did not appear to be the source of the amyloid.

### Etiologic Concepts

The apparent common denominators of amyloidosis of the heart are senility and malnutrition. The decrease of body weight, the reduced blood hemoglobin and erythrocytes, and the small livers in several patients of the present series have been noted above. From the clinical information available, malnutrition was caused by restricted food intake (14 cases), impaired absorption of food related to coexistent gastrointestinal lesions (6 cases), and faulty utilization of food related to the presence of a cancer (3 cases). Bock (1948)[6] studied the serum proteins of 41 normal men and 35 normal women 60 to 95 years of age. He found that the albumin decreased 0.3 to 0.4 gm. per 100 cc. and that the globulin rose 0.16 to 0.21 gm. per 100 cc. in subjects over the age of 80 years, compared to the same values for those under this age. He attributed these changes to failing regulation of appetite and a reduced capacity for synthesis of plasma proteins. In the present series, restricted food intake, impaired absorption of food, and faulty utilization of food in several patients could have exaggerated these tendencies in the levels of blood albumin and globulin. The blood proteins were studied in only six cases. Total protein ranged between 4.4 and 6.5 gm. per 100 cc. Of three cases, in which they were fractionated, two showed decreased albumin and relatively elevated globulin. No electrophoretic studies were recorded. In a case of amyloidosis of the heart and several other sites, van der Straeten et al. (1954)[66] observed a total protein of 6.60 gm. per 100 cc., which by electrophoresis was distributed as follows: albumin, 1.88 gm.; $\alpha_1$-globulin, 0.69 gm.; $\alpha_2$-globulin, 1.21 gm.; $\beta$-globulin, 1.12 gm., and $\gamma$-globulin, 1.69 gm. This type of investigation might prove fruitful in assessing cases of cardiac amyloidosis. Ranström (1946)[52] mentioned hyperglobulinemia as part of the picture of cardiac amyloidosis but gave no analyses to support this contention. The figures for blood albumin and globulin given by Hulbert and Meyer (1949)[26] and Jackson and Slavin (1954)[29] for their cases and by Thomashow et al. (1953)[64] for their Case 3 showed no significant disturbance of these values.

From the foregoing, it is seen that aging and malnutrition can contribute in some cases to lowered albumin and relatively or even absolutely increased blood globulin, a well-known accompaniment of many cases of amyloidosis resulting from chronic suppuration or multiple myeloma. The decreased albumin by itself could contribute to lowered blood pressure and lowered osmotic pressure, and so fluid containing globulin could leak into the stroma of the myocardium and become hardened in the form of amyloid. Likewise, hypoalbuminemia could be part of the explanation for the edema of the lungs, hydrothorax, and peripheral edema observed in cases of cardiac amyloidosis, in which heart failure is so important. Another facet of protein depletion, which in dogs was related to the production of gastric ulcers by Hahn et al. (1957),[21] concerned the chronic duodenal ulcer of Cases 6 and 15 and possibly the acute cholecystitis in Case 5.

Malnutrition was probably also responsible for reduced availability of vitamins of the B-complex, notably thiamine and ascorbic acid (vitamin C). In normal men and women, Kirk and Chieffi (1949)[36] observed blood thiamine values as follows: 48 subjects 60 to 69 years of age, 3.7$\mu$g.; 71 subjects 70 to 79, 3.2$\mu$g., and 59 subjects 80+, 3.1$\mu$g., indicating a slight reduction of this vitamin in patients over 70 years of age, partly due to the greater frequency of anemia with age, since erythrocytes contain more thiamine than plasma. They also

observed values below 2μg. in 8% of subjects 60 to 69 years of age; in 10%, 70 to 79, and in 12%, 80+. These authors (1953)[37] also determined ascorbic acid values in a group of 61 men and 81 women 40 to 103 years of age. In the men, the curve for ascorbic acid was as follows: 40 to 59 years of age, 0.59 mg.; 60 to 69, 0.42 mg.; 70 to 74, 0.41 mg.; 75 to 79, 0.35 mg., and 80 to 103, 0.33 mg. In the women, the curve was as follows: 40 to 59 years of age, 0.48 mg.; 60 to 69, 0.42 mg.; 70 to 74, 0.43 mg.; 75 to 79, 0.41 mg., and 80 to 103, 0.40 mg. They concluded that blood ascorbic acid declines significantly with age in men but not in women. They also quoted values obtained by Hagtvet, in Norway, in 722 normal persons 20 to 50 years of age and in 89 hospital patients 60 years of age and older. In the normal group, the values for ascorbic acid in April, May, June, August, September, and October were 0.22 mg., 0.21 mg., 0.16 mg., 0.93 mg., 0.89 mg., and 0.87 mg., as compared to 0.05 mg., 0.05 mg., 0.05 mg., 0.19 mg., 0.27 mg., and 0.45 mg. for the elderly patients. The known occurrence of edema, notably in the stroma of the heart, in patients with thiamine deficiency and the possibility that ascorbic acid deficiency could cause edema only in the same location, in contrast to the hemorrhage observed in frank scurvy, would suggest that such deficiencies could be operative in the leakage of globulin-rich fluid through the capillaries into the stroma of the myocardium in patients, who are also likely to have the blood protein imbalance described above. The differential drop of ascorbic acid with age in men might be a factor in explaining their susceptibility to cardiac amyloidosis. Contributing social factors are discussed by Sinclair (1957).[59]

From the foregoing, the concept that amyloidosis is ever primary is doubtful. When this process is fully evaluated, the conclusion seems tenable that amyloidosis will be proved secondary only with the basis a disturbance in blood proteins, specifically an imbalance favoring a relative or an absolute hyperglobulinemia in addition to vitamin deficiencies and other possible factors not discussed or as yet unappreciated.

Certain recommendations for the study of cases of amyloidosis of the heart may be made. On the clinical side, careful analysis of diet and serial observations of blood pressure, of blood proteins (chemical and electrophoretic fractionation), of urine albumin, and of electrocardiograms are indicated. On the pathologic side, mapping of the distribution of amyloid in the myocardium with especial reference to the conduction system is desirable.

## Summary and Conclusions

Among 1150 autopsies of persons 70 years of age and older between 1940 and 1955 at Colorado General Hospital, 17 cases (1.5%) of amyloidosis of the heart not explained by chronic suppuration or multiple myeloma occurred in 16 men and 1 woman (corrected ratio of 6.4:1), all but 2 of whom were 80 years of age and older. The sex and age preponderance in cardiac amyloidosis was amply confirmed in the literature.

Amyloidosis of the heart caused death in seven patients, cooperated in causing death in two, and contributed variably to death in eight.

Symptoms and signs of cardiac failure not responding readily to treatment were common. The chest radiograph revealed variable heart size, pulmonary edema, and hydrothorax. Important findings in the electrocardiograph were low voltage of ventricular complex, low amplitude of atrial complex, delayed intraventricular conduction, and partial atrioventricular block.

Amyloid, appearing most frequently as gross granules in the atrial endocardium and as granules or diffuse infiltrations of the myocardium of all chambers, was less common in the epicardium and valves. The hearts affected ranged from normal size to striking hypertrophy. Coronary arteriosclerosis was usually minimal to moderate and nonocclusive.

The amyloid, deposited in the myocardium, predominantly among the muscle fibers and the capillaries, was metachromatic with methyl violet or crystal violet, variably red with Congo red, and a distinctive shade with ammoniacal silver. Reticulum was surrounded by amyloid. In one study, the amount of amyloid was progressively severer with age.

The apparent common denominators of amyloidosis of the heart, namely, senility and malnutrition, encompassed a consideration of (1) weight loss, anemia, poor dentition, and atrophy of the liver; (2) restricted intake, impaired absorption, and faulty utilization of food; (3) the progressive decline of blood albumin, the rise of blood globulin, and the fall of thiamine with age in both sexes, and (4) the progressive decline of blood ascorbic acid with age in men.

Amyloidosis, regardless of localization, is properly thought of as secondary on the basis of a disturbance of blood proteins, in which hyperglobulinemia is paramount. Other factors as yet unappreciated may also be operative.

Study of the blood proteins by electrophoresis and an accurate mapping of amyloid in the heart in relation to the conduction system are desirable in future investigations of amyloidosis of the heart.

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# Spontaneous Regression in Rabbit Intraocular Atheromatous Aortic Homotransplants

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Intraocular homotransplants of normal and atheromatous rabbit aorta have been maintained successfully for more than a year in healthy untreated hosts. The operative technique and preliminary observations have been reported.[1,2]

The principal purpose of this paper is to report our observations on the apparent sequence of histologic and histochemical changes in the intima of spontaneously regressing atheromatous transplants maintained in the anterior eye chambers of healthy untreated host rabbits.

## Materials and Methods

Plaques were developed in the aortas of donor animals by feeding an atherogenic diet consisting of 90 parts rabbit chow, 9 parts cottonseed oil, and 1 part cholesterol. After about three months, a donor was killed and a uniformly atherosclerosed area of aorta was selected and divided into portions of approximately 4 sq. mm. A representative piece was taken as a control. The others were transplanted, with the intimal surface facing outward, into the anterior eye chambers of anesthetized healthy littermates. Host animals were killed at selected intervals (usually one every four months), and the transplants were removed for histologic and histochemic study. Normal rabbit aorta was transplanted in the same manner, except that the hosts were not always littermates. Transplants and control pieces of aorta were divided into two portions. Frozen sections were cut of one portion, mounted unstained for study with the polarizing microscope, or stained with oil red O. Paraffin sectins were made of the other portion for staining by hematoxylin and eosin and other techniques.

Two groups of host animals were used in this study: (1) a group with transplants of atheromatous aorta and (2) a group with transplants of normal aorta. Group 1 consisted of four female littermate New Zealand White rabbits. The donor was the fifth female of the same litter and had been maintained with the atherogenic diet ad libitum for 98 days. Hosts were 6 months old at the time the transplants were made, and the animals were killed at 4, 8, 10, and 16 months thereafter.

Group 2 animals were used for a parallel study for comparison with those of Group 1. The donor animal was a male New Zealand White rabbit approximately one year of age. The host animals were two male and one female New Zealand Whites, and one female New Zealand White-Checker cross, from three different litters. The animals were killed at 4, 8, 10, and 12 months postoperatively.

The donor animal in Group 2 and all the host animals in both groups were maintained throughout life with ad libitum diets of rabbit chow.

## Results

Recovery of the host animals from the operation was prompt and uncomplicated by infection. Gross observation showed that all of the transplants became attached to the anterior surface of the iris by fibrous tissue in less than eight days. Within 30 days, several clearly visible blood vessels could be seen extending from the iris of the host to the adventitia of the transplants. The intima of the atheromatous transplants became extensively vascularized, but no vascularization of normal intima occurred. After eight months, the intima of the atheromatous transplants, when viewed in situ with the microscope in reflected light, had a glittering appearance suggesting the presence of numerous small light-reflecting crystals.

Submitted for publication Dec. 2, 1957.

This investigation supported by U. S. P. H. S. Grants H-2302 and H-3207(C).

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Fig. 1. — Photomicrograph of section of control piece of rabbit aorta, showing closely packed foam cells in the thickened intima and reduplicated internal elastic membrane. Hematoxylin and eosin; reduced about 15% from mag. × 78.

This glittering was not observed in newly transplanted pieces of such aorta or in transplants of normal aorta.

The thickened intima of the atheromatous control piece consisted mostly of a thick mass of foam cells. Splitting and/or reduplication of the internal elastic membrane had already occurred (Fig. 1). No cholesterol crystals were observed, but numerous evenly distributed globules of material were present which exhibited the cruciform birefringence indicative of cholesterol esters.

Indications of postoperative histologic and histochemic changes were seen in sections of 4-, 8-, 10-, and 16-month-old atheromatous transplants. Especially noticeable was a progressive decrease in the number of foam cells and the sudanophilic material. Fibrous tissue became more abundant throughout the intima, especially in the outer free zone. The surface fibroelastic layer became thicker as the transplants became older and eventually appeared to form a capsule-like structure (Fig. 2). Acicular clefts between foam cells were apparent at the end of four months (Fig. 3) and were still present in the intima of 8-, 10-, and 16-month-old transplants. Crystals of choles-

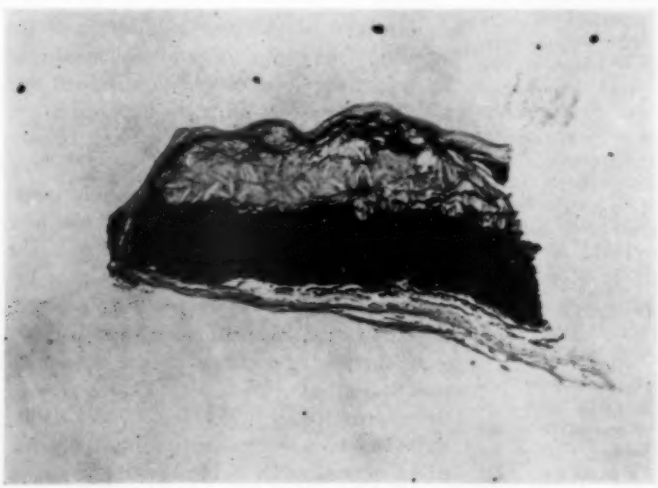

Fig. 2. — Photomicrograph of section of 10-month-old transplant of atheromatous rabbit aorta, showing fibroelastic capsule, reduplicated internal elastic membrane, and acicular cholesterol clefts. A portion of the host's iris is beneath the transplant. Verhoeff's stain reduced 8% from mag. × 50.

Fig. 3. — Photomicrograph of section of 4-month-old transplant of atheromatous aorta, showing acicular cholesterol clefts and foam cells in the intima. Indications of calcification were evident in the darkest areas. A small piece of the host's cornea is at the upper left. Hematoxylin and eosin; reduced about 15% from mag. × 78.

terol were present at four months (Fig. 4) and were still present in the older transplants. During the same period of time, the amount of globular material with cruciform birefringence progressively decreased. In the 16-month transplant, virtually none of the globular material could be detected, but cholesterol crystals were prominent, especially in the deeper parts of the transplant. Comparison of unstained with stained sections revealed a positive correlation between the number of crystals in the former and the clefts in the latter. These changes also corresponded in time with the occurrence of the glittering appearance seen in the transplants in situ.

There were progressive changes in the amount and distribution of sudanophilic material. Instead of the dense rather uniform packing of the intima with small droplets of lipid seen in the control piece, sudanophilic material became restricted mostly to small areas around the cholesterol crystals and became progressively less in amount.

Only traces of necrosis were seen in some of the sections of transplants. A few minute

Fig. 4.—Photomicrograph of section of 4-month-old transplant of atheromatous aorta, showing in the intima crystals of cholesterol and globules exhibiting the cruciform birefringence indicative of cholesterol esters. Frozen section, polarized light; reduced about 15% from mag. × 78.

spots of calcification were seen in the deepest parts of the lesions adjacent to the media, particularly in the older transplants. When these small spots of calcification were present, their distribution did not always coincide with that of the cholesterol crystals. In most sections where crystals were present, no indication of calcification was seen.

In the parallel study of four host rabbits with transplants of normal aorta, little gross change was observed in the basic morphology of the transplants over a 12-month period. The intima remained thin. The elastic lamellae retained their identity but appeared to be a little more widely separated in the older transplants. The orientation of the nuclei of many of the smooth muscle cells in older transplants indicated that these cells now extended at an angle from one lamella to another instead of lying parallel between adjacent lamellae.

## Comment

The apparent sequence of histologic and histochemic changes which occurred in the intima of transplanted atheromatous aorta seems to be similar to that described by Anitschkow [3] in rabbit aorta after cessation of cholesterol feeding. Our observations lead us to agree with Anitschkow that regressive as well as progressive changes occur. Since we found evidence of conversion of cholesterol esters to crystalline cholesterol and the formation of additional fibroelastic tissue in transplants postoperatively, we believe these changes should be classified as regressive.

Because various tissue and physicochemic changes occur during plaque formation, the regressive processes may not necessarily be identical with, or reversals of, progressive processes. At least some of the regressive processes seem to occur in a series of steps, and perhaps the complex of factors influencing one step may be without the same effect on others.

According to Ophüls,[4] some investigators think that cholesterol crystal formation in atheromatous lesions is preceded by necrosis. We have seen crystals in transplants in which no evidence of necrosis was detected. When both were present in the same section, crystals were present where no indications of calcification were seen.

Transplantation of pieces of atheromatous arteries into the anterior eye chambers of host animals of the same species seems not only to be a valid method for the study of regression but in addition appears to have certain advantages. Transplants of a single plaque of known features may be made into the anterior eye chambers of several animals, and these animals may be subjected to the same or different experimental procedures. Comparisons may be made of the degree of effectiveness of the procedures and of the time required for each of the regressive changes to occur in a given plaque. The technique is of further advantage in that the atherogenic processes are minimized in untreated hosts, and thus a better opportunity is provided to identify the histologic and histochemic processes which occur during regression and to study the biophysicochemical factors influencing each.

## Summary and Conclusions

Normal and atheromatous aortic homotransplants have been maintained successfully in the anterior eye chambers of host rabbits for over a year. The adventitia of both types of transplants becomes vascularized by blood vessels from the iris of the host, but the intima becomes vascularized only in the atheromatous transplants. Indications of sequential regressive processes were observed in atheromatous transplants in untreated healthy hosts. These consisted of a reduction in the number of foam cells and their replacement by fibrous connective tissue, the conversion of cholesterol esters to cholesterol crystals with the formation of acicular clefts between the cells, and a decrease in the amount of sudanophilic material. Similar changes have been reported by other investigators to occur in the plaques

of intact aortas of animals after removal from atherogenic diets.

Transplanting plaques from an animal in which they have been developed into healthy host animals immediately minimizes the atherogenic processes and permits easier identification and study of the regressive processes. The method, therefore, appears to be a valid and an advantageous one for studying regression of experimental cholesterol atheromatosis.

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# Studies on the Pathogenesis of Adrenal-Regeneration Hypertension in the Rat

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In 1955, Skelton disclosed that rats became hypertensive and developed widespread vascular lesions after adrenal enucleation, unilateral nephrectomy, and the administration of 1% saline in the drinking water.[1] These findings have been amply confirmed by us and by other workers.[2-4] However, the suggestion that the regenerating adrenal cortex plays a direct etiologic role in the genesis of adrenal-regeneration hypertension has not been fully corroborated; furthermore, the statement has been made that "no hypertensive principle is secreted by the regenerating adrenal nor is the hypertension a result of an imbalance in adrenal cortical activity."[5]

Ingle and Higgins, in 1938, showed that if one adrenal of the rat is enucleated and eight weeks later the contralateral adrenal is removed, then regeneration of cortical tissue will still ensue from the adrenal remnant.[6] This technique, we felt, if applied to a study of adrenal-regeneration hypertension, might allow a more direct approach to the relationships between the development of hypertension and actual adrenal regeneration. The details of such an experiment, and others which add to our knowledge of the pathogenesis of adrenal-regeneration hypertension, are the subject of this paper.

## Methods

The rats used in this study were male animals of the Wistar strain, weighing between 80 and 90 gm. They were maintained on a commercial rat food and tap water; after the various operative procedures, the tap water was replaced by 1% saline. The operative techniques have been described in detail elsewhere.[8] After three control readings of the systolic blood pressure were taken, by the photoelectric method of Kersten,[7] the animals were anesthetized with pentobarbital sodium for surgical operation. The groups, each composed of 25 animals (with the exception of Group IV, which included 30 animals), were treated as follows: Group I, bilateral adrenalectomy and right unilateral nephrectomy; Group II, right unilateral nephrectomy and right unilateral adrenalectomy; Group III, right unilateral nephrectomy only, and in Group IV the animals were unilaterally nephrectomized on the right side and the right adrenal gland was enucleated as described by Ingle. Five weeks later the contralateral adrenal was removed from the animals of this group.

Blood pressures were measured three times weekly on each animal; these values were pooled to obtain the mean weekly blood pressure for the group. Five animals from Group IV were killed at the time of the second operation, the remaining animals from all groups were killed, with use of chloroform, at the end of a 10-week experimental period. The tissues were removed and, after adherent fat and connective tissue was dissected away, weighed fresh on a Roller Smith Torsion balance. The following organs were examined histologically after fixation in Bouin's solution: brain, heart, kidney, pituitary, thyroid, pancreas, stomach, intestine, mesentery, liver, and spleen. Hematoxylin and eosin was used as a routine stain for all organs. Kidneys, and in some cases heart and mesentery, were stained with Cason's trichrome[8] and Sudan IV in frozen section. After fixation frozen sections were made of the adrenal gland and the remaining tissue was embedded in paraffin for hematoxylin and eosin staining. Frozen sections were examined for birefringent material, and additional slides were stained with Sudan IV and hematoxylin.

## Results

*I. Blood Pressure.*—It is evident from Figures 1 to 4 that a sustained rise in systolic blood pressure occurred after the "sensitization" of the animals by unilateral

Submitted for publication Dec. 23, 1957.

From the Research Department, Ayerst, McKenna and Harrison Ltd.

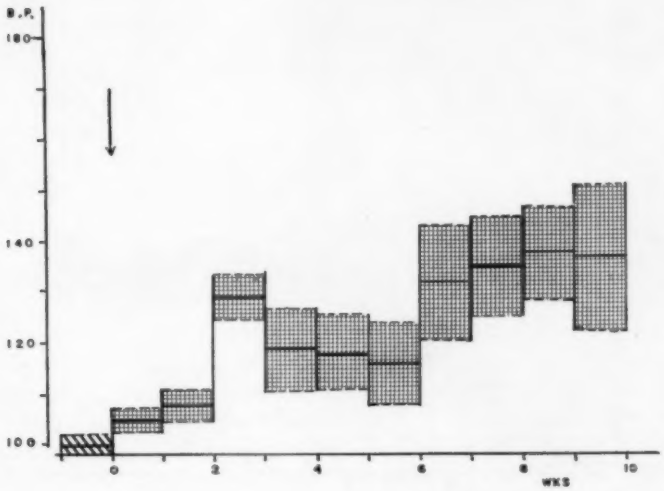


Fig. 1 (Group I).—Blood pressure values. Horizontal lines represent mean weekly blood pressure, and the shaded areas, the 95% confidence limits. At the arrow, bilateral adrenalectomy and right unilateral nephrectomy.

nephrectomy and the addition of 1% sodium chloride to the drinking water. However, since only systolic blood pressures above 150 mm. of mercury are generally considered as hypertensive,[12] one must conclude that hypertension did not ensue in any of the groups after the "sensitization" procedure and bilateral adrenalectomy, unilateral adrenalectomy, or unilateral adrenal enucleation. Similarly, Group III, in which the adrenals were left intact, failed to show elevations of the blood pressure to hypertensive levels. After four to five weeks, the blood pressure became stabilized at levels below 140 mm. of mercury in all groups, and in Groups I, II, and III these levels were maintained till the end of the experiment. After the second operation of the animals of Group IV, removal of the intact adrenal gland, the mean blood pressure of these animals became elevated to levels which are clearly hypertensive. This elevation of blood pressure, from levels of 136 to 182 mm. of mercury, oc-

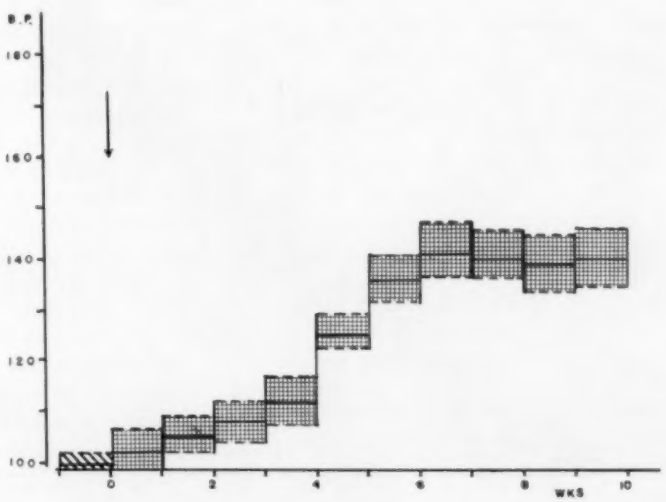


Fig. 2 (Group II).—At arrow, right unilateral nephrectomy and right unilateral adrenalectomy.

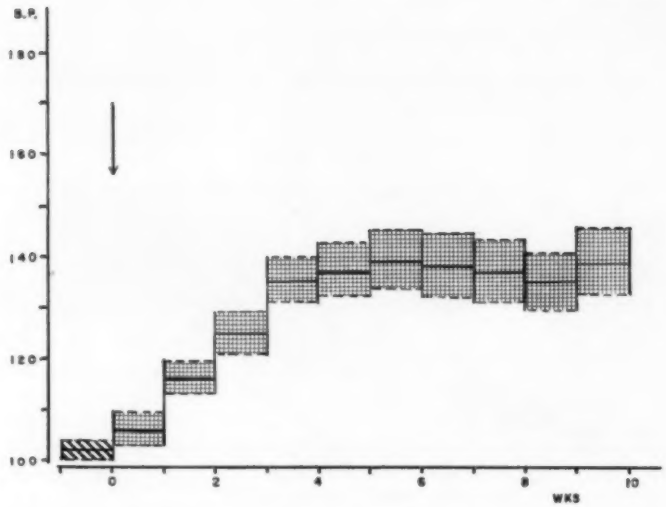


Fig. 3 (Group III).—At the arrow, right unilateral nephrectomy.

curred during the period when, as will be shown later, there was regeneration of cortical tissue from the adrenal remnant.

*II. Body and Tissue Weights.*—The average body weights of the animals at the time of killing were as follows: Group I, 298 gm.; Group II, 328 gm.; Group III, 333 gm., and Group IV, 346 gm. The relatively poor growth of animals which are bilaterally adrenalectomized is well known and accounts for the lower mean body weight of the animals from Group I.

The organ weights of the animals in each group are given in the Table. It is not possible to compare the weights of the adrenal glands from the animals of the various groups. The single adrenals from the animals of Group II have undergone compensatory hypertrophy and are much greater in weight than a single adrenal from the animals of Group III, in which the adrenals remained intact. At the time of the second operation on the animals of Group IV, the

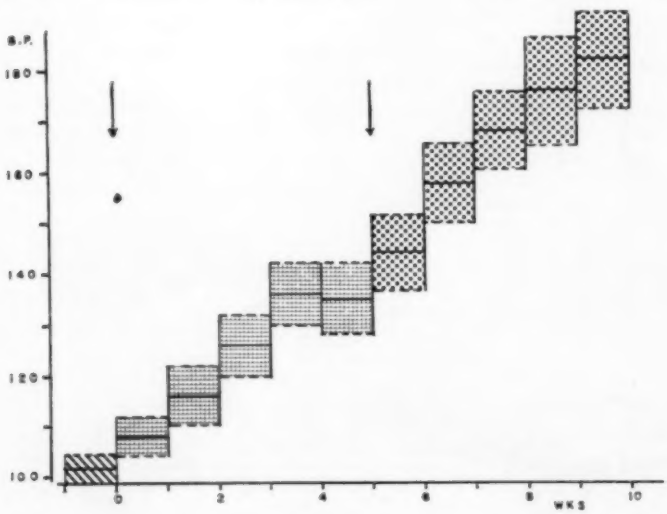


Fig. 4 (Group IV).—At the left arrow, right nephrectomy and right adrenal enucleation. At the right arrow, the contralateral intact adrenal was removed.

TABLE 1.—*Tissue Weights*

| Tissue | Group I: Bilateral Adrenalectomy & Unilateral Nephrectomy | Group II: Unilateral Adrenalectomy & Unilateral Nephrectomy | Group III: Unilateral Nephrectomy | Group IV: Unilateral Adrenal Enucleation & Nephrectomy, Delayed Contralateral Adrenalectomy |
|---|---|---|---|---|
| Adrenal(s), mg. | — | 39.5±1.0 | 49.4±1.5 | 32.5±1.9 |
| Thymus, mg. | 406±3.4 * | 340±16 | 372±24 | 327±18 |
| Thyroids, mg. | 20.9±1.4 | 23.9±0.9 | 21.5±1.0 | 24.2±0.9 |
| Pituitary, mg. | 11.6±0.6 | 11.1±0.03 | 10.2±0.3 | 11.5±0.4 |
| Spleen, gm. | 0.706±0.06 | 0.657±0.02 | 0.639±0.02 | 0.701±0.02 |
| Brain, gm. | 2.015±0.05 | 1.831±0.02 | 1.835±0.03 | 1.895±0.03 |
| Liver, gm. | 13.8±0.9 | 13.6±0.03 | 14.30±0.5 | 16.6±0.5 |
| Heart, gm. | 0.974±0.08 | 1.019±0.02 | 1.021±0.03 | 1.195±0.04 |
| Kidney, gm. | 1.849±0.12 | 2.117±0.05 | 2.196±0.05 | 2.631±0.08 |

* Significant at the 95% confidence limit compared to Group III.

contralateral adrenals, which were removed intact, had a mean weight of 32.8±2.1 mg. The adrenal remnants from the five animals of this group which were killed at this time weighed less than 0.5 mg. In only four of these animals was it possible to recognize adrenal cortical tissue by histological examination. At the end of the experiment the regenerated adrenal glands of these animals had a mean weight of 32.5±1.9 mg. Thus, the regenerated adrenal cortical glands were greater in size than a single gland from the animals of the intact group. The thymus glands of the animals of Group I show a significant hyperplasia; this would be expected following bilateral adrenalectomy.

In the Table a depression in thyroid weight is shown for the adrenalectomized animals of Group I. When the thyroid weights are calculated in terms of body weight, this difference is no longer evident, and thus the smaller weight of this gland may be attributed to an over-all decrease in body weight. No differences were observed in the weights of the pituitary gland, spleen, and brain of the animals from the various groups. The liver weights of the animals of Group IV were significantly greater than those of the remaining groups; passive congestion in the livers of these animals, it is felt, accounts for this increased weight. The heart and kidney weights of the animals from Group IV are significantly greater than the weight of these organs from the animals of the other groups. This renal and cardiac hypertrophy would be anticipated on the basis of the high blood pressure of this group and, in fact, indirectly confirms the presence of hypertension.

*III. Gross and Microscopic Pathology.*—Gross examination of the animals from each group at autopsy, and microscopic study of the individual organs, did not reveal any evidence of pathology in the brain, pancreas, stomach, intestine, spleen, and pituitary gland.

Two animals from Group II and one animal from Group IV showed evidence of acute bronchopneumonia at autopsy; however, this is a casual finding in the animals from our colony, and it is felt to be unrelated to the experimental conditions. A total of nine animals from the various groups died of pneumonia during the experimental period.

The lesions detected in the other organs are described below according to the experimental group.

Group I: A total of 12 animals from this group died during the experimental period. Three of these animals died while restrained in the apparatus for blood pressure determination. These animals, compared to those in the other groups, were particularly sensitive to this stress. At autopsy, four animals were found to have regenerated adrenal cortical tissue; it was impossible to determine whether this was the result of incomplete adrenal extirpation or hypertrophy of accessory adrenal tissue. One animal was found to have a severe hydronephrosis. These five animals were excluded from the group. One animal from this group had a mild chronic focal pyelonephritis. With the exception of three ani-

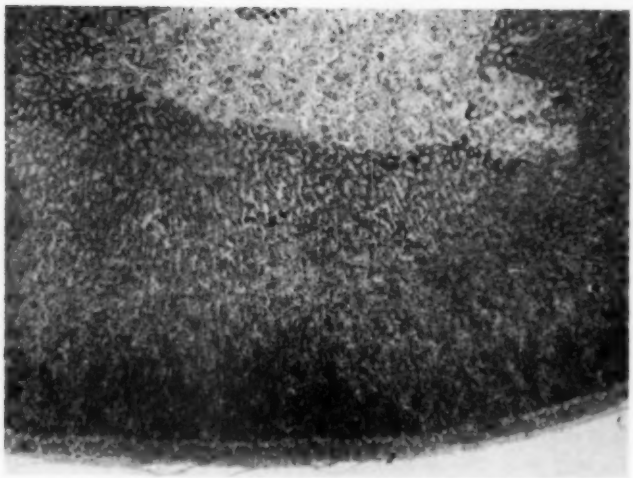

Fig. 5. — Adrenal cortex after unilateral adrenalectomy. Wide cortex, with thickening of the zona fasciculata. Sudan IV and hematoxylin; × 40.

mals, which had a slight interacinar fibrosis, the thyroid glands were well preserved. No lesions were detected in other organs, and no evidence of vascular lesions was found.

Group II: The only lesions found in the animals of this group were in the kidney. In two rats there were scattered foci of round cells without alterations in the renal parenchyma or evidence of vascular changes.

The single adrenal glands from the animals were enlarged and brownish-yellow. The capsule was thick, with the zona glomerulosa of normal thickness, but the cells were darkly stained and poor in lipoid material. The zona fasciculata was wide and heavily infiltrated with lipoid material; this lipoid infiltration was most concentrated at the periphery of this zone. The zona reticularis was normal in appearance (Fig. 5).

Group III: Three animals from this group which had blood pressures above the average were found to have a moderate degree of chronic pyelonephritis without

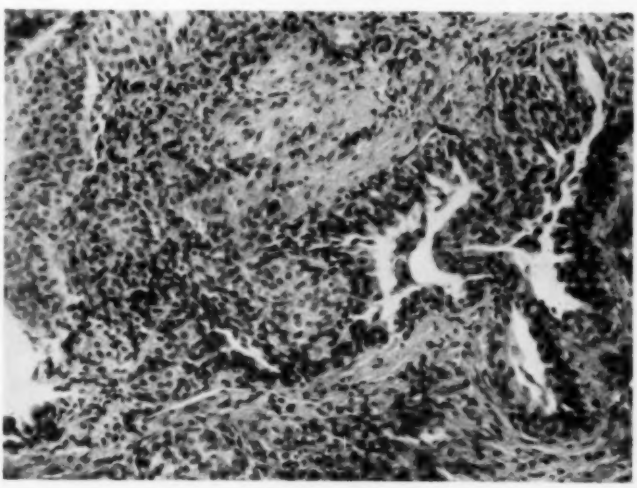

Fig. 6.—Adrenal remnant, five weeks after enucleation. Solid bundles and some glandular structures, composed of darkly stained small cells, are visible embedded in dense connective tissue. Hematoxylin and eosin; × 200.

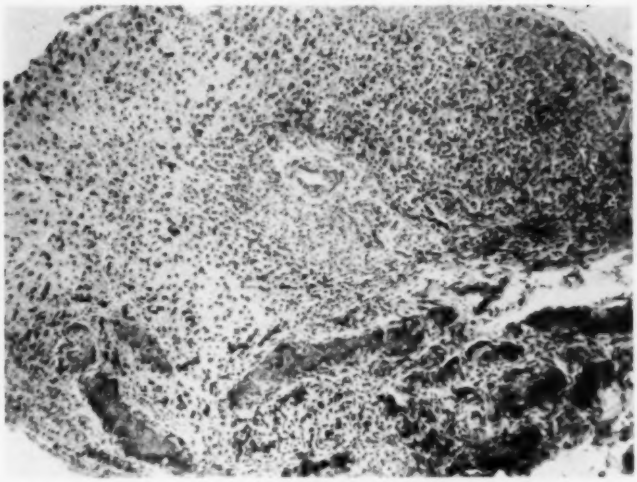

Fig. 7.—Adrenal remnant, five weeks after enucleation. The weight of this remnant was 0.05 mg. Note the marked fibrosis and calcification. Only a few small areas of atrophic cortical tissue remained. Hematoxylin and eosin; × 110.

vascular involvement. No changes were detected in the other organs.

Group IV: At the time of the second operation of the animals of this group, the adrenal remnants from the five animals which were killed and the adrenal glands which were removed from the remaining animals were preserved for examination. The intact adrenals from these animals were enlarged and microscopically resembled the adrenals from the animals of Group II. However, one difference was noticed in that there appeared to be an over-all decrease in lipoid content in the zona fasciculata.

The adrenal remnants varied from small nests of undifferentiated cortical cells to circumscribed groups of cells which showed no zonal differentiation (Fig. 6). Fibrous tissue proliferation was evident in each remnant, with bands of connective tissue, occasionally calcified, separating the cortical cells. The vessel stems had thickened walls and narrowed lumens (Fig. 7).

At the end of the 10-week experimental period, adrenal regeneration could be recognized in all rats. The general architecture of these adrenals showed a marked nodularity (Fig. 8). For the most part, the adrenal cortical cells showed a clear zonal

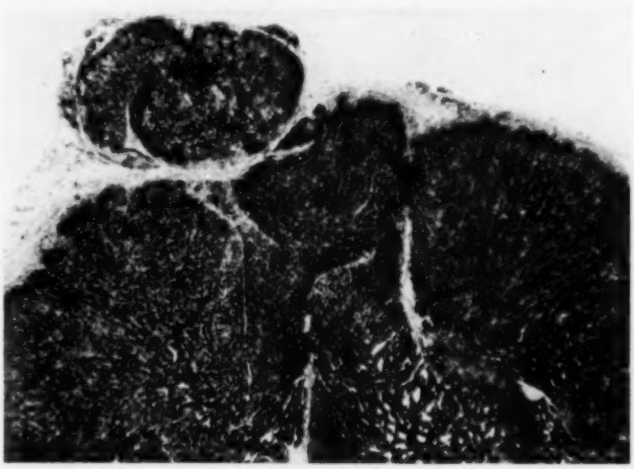

Fig. 8. — Adrenal cortex. Delayed regeneration. Note the marked nodular structure and zonal differentiation. Sudan IV and hematoxylin; × 40.

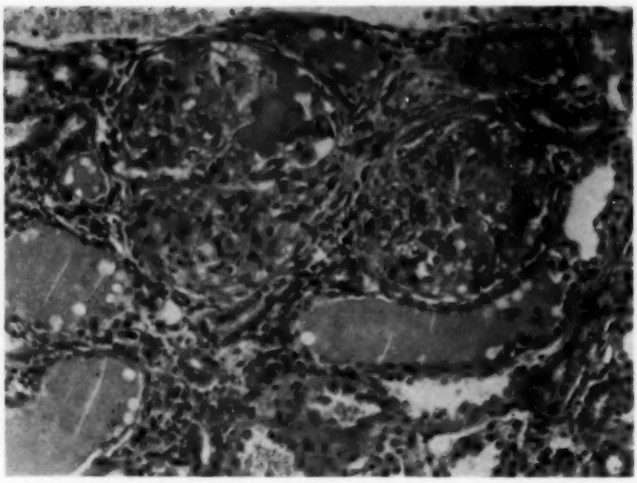

Fig. 9.—Kidney in delayed regeneration. Marked enlargement of the glomeruli. Focal necrosis of the glomerular loops and thickening of the basement membrane. Proliferative endarteritis of the afferent vessels. Dilated tubules, lined with flattened cells, containing protein casts. Hematoxylin and eosin; × 175.

differentiation, with an increase in the lipoid and double-refractive material of the zona fasciculata and reticularis. However, in many areas, small islands of atrophic darkly stained cortical cells surrounded by thick fibrous tissue were seen. The central portions of most of the regenerated adrenals were occupied by connective tissue masses containing calcified bundles and foreign-body giant cells.

The kidneys from these animals were enlarged, particularly so in four animals which had kidney weights much greater than the average. In these latter animals, the capsule stripped easily and the renal surface was smooth. On the cut surface one could recognize small, yellow, irregular and indistinct areas. One animal showed a marked hydronephrosis. On microscopic examination, 10 animals had some arteriolar and glomerular lesions. Three of these were particularly severe, with arteriolar nephrosclerosis and generalized vascular necrosis. The glomeruli in these animals were enlarged and bloodless, with focal homogenization of the tufts and occlusion of the lumina (Fig. 9). Adhesions were present between the glomerular tufts and Bow-

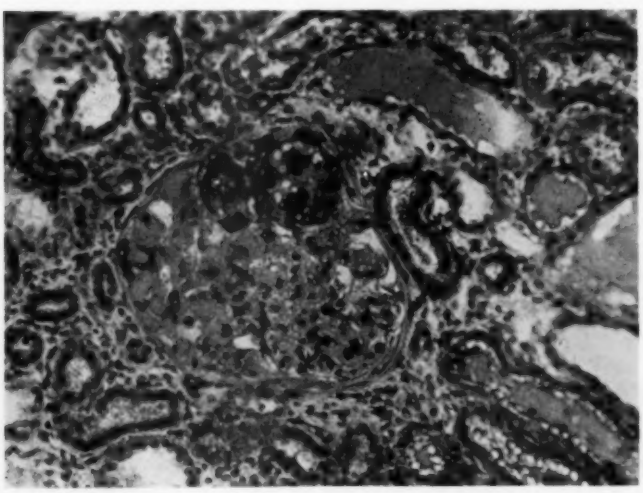

Fig. 10.—Lipoid deposition in necrotic glomeruli. This lipoid was double-refractile. Sudan IV and hematoxylin; × 175.

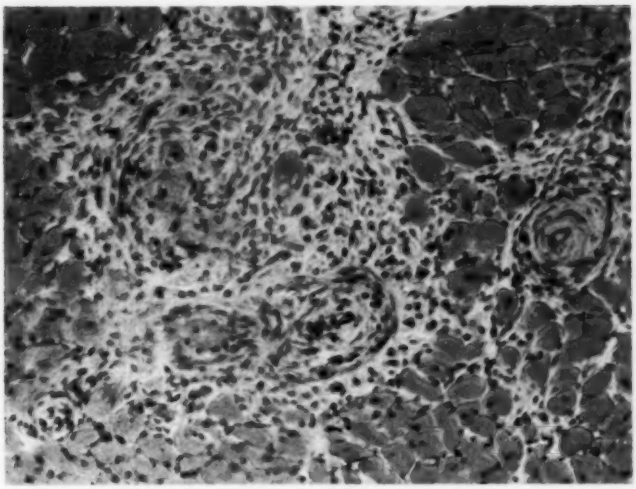

Fig. 11. — Productive endarteritis and scars in the myocardium. Note the occlusion of some of the arterioles. The darkly stained streaks in the arterioles represent lipoid deposition. Sudan IV and hematoxylin; × 175.

man's capsule, and proliferation of the epithelium of the capsule formed crescents around the glomeruli. Only a few glomeruli remained intact. The walls of the afferent vessels and smaller vessels were thickened, and focal homogenization of the walls, proliferation of subendothelial cells, and occlusion of the lumen were present. Fatty infiltration with double-refractive material was seen in necrotized glomerular loops and in the wall of the vessels involved (Fig. 10). The epithelium of the proximal convoluted tubules was swollen and granular, containing fine lipoid droplets. There was focal dilatation of nephrons, which were lined by flattened darkly stained cells and had protein-hyalin cylinders in their lumen. The interstitium of the kidney was edematous, and small scattered foci of lymphocytic infiltration were seen in the subcapsular areas of the cortex. The mucosa of the pelvis was well preserved, with edematous swelling in the submucosa. These changes were qualitatively similar but more moderate in the remaining animals.

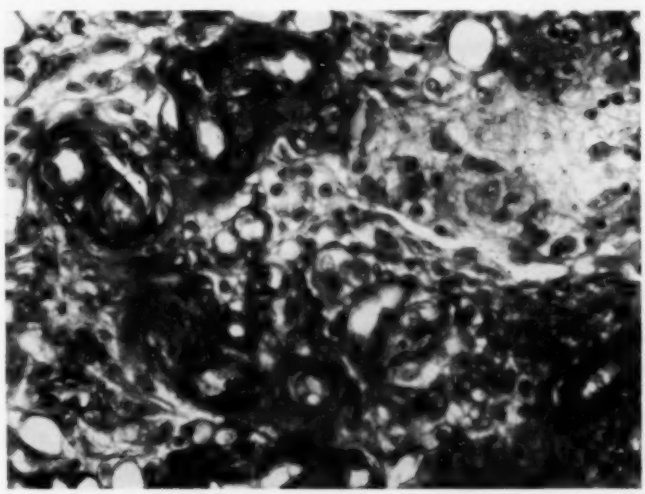

Fig. 12. — Endarteritis and fibrous thickening of the capillaries of the mesentery. Cason's trichrome; × 300.

The hearts of two animals showed transverse yellowish streaks in the gross. Microscopically, these were areas of myocardial scar formation and necrosis of the muscle fibers. These myocardial changes were the result of obliterative endarteritis and necrosis of smaller branches of the coronary arteries (Fig. 11). These vascular changes were manifested by focal fibrinoid or hyaline homogenization, swelling of the walls, proliferation of subintimal cells, and perivascular inflammatory reaction. Changes similar to those found in the vessels of the heart were present in the vesels of the mesentery (Fig. 12) and pancreas of these rats.

## Comment

The treatment of the experimental groups was similar in that all animals were unilaterally nephrectomized and given 1% sodium chloride in the drinking water. The responses of each group to this procedure were also alike in that moderate increases in blood pressure were seen but with failure to rise to hypertensive levels or to develop cardiovascular or renal hypertrophy and lesions. Thus, adrenal insufficiency following bilateral adrenalectomy, adrenal hypertrophy following unilateral adrenalectomy, or adrenal enucleation per se failed to influence the blood pressure, and the levels were comparable to the group in which the adrenal glands were left intact. These results are in agreement with the findings of many other workers who have demonstrated that the administration of excessive sodium chloride to the rat, with or without unilateral nephrectomy, is sufficient to cause elevation of the blood pressure.[9-11] However, adrenal regeneration following removal of the intact adrenal from the animals which had an enucleated adrenal on the opposite side resulted in regeneration of the adrenal remnant and the development of marked hypertension, renal and cardiac hypertrophy, and vascular lesions. These results support the thesis that adrenal regeneration plays a primary role in the development of this hypertension. We have found, as shown by Ingle and Higgins,[6] that after adrenal enucleation the adrenal remnant, consisting of capsule and adherent cortical cells, survives over a period of five weeks and is capable of regeneration when the contralateral adrenal is removed. The histological picture of these adrenals does not differ significantly from that in experiments in which the adrenal enucleation and contralateral adrenalectomy were performed simultaneously.[3] A minor exception to this might be a somewhat greater nodularity noted in the adrenals from the animals with delayed regeneration. Furthermore, these studies show that such delayed regeneration in unilaterally nephrectomized animals can provide the stimulus for the development of hypertension.

Any discussion on the essential nature of the role of the adrenal cortical cells in the pathogenesis of adrenal-regeneration hypertension must at this stage of our knowledge be purely speculative. One might theorize that excessive mineralocorticoids from regenerating adrenal cortical cells were an etiologic factor. However, a number of findings do not support such a theory. Skelton [1] has failed to demonstrate a significant alteration in serum electrolytes during the development of adrenal-regeneration hypertension. In these experiments and in our previous work we have failed to find any histological changes in the glomerulosa zone, which is generally conceded to be the site of steroidogenesis of aldosterone in the rat. Similarly, Amphenone (1,2-bis-[*p*-aminophenyl]-2-methyl propanone-1), an adrenal cortical depressant which prevents the development of adrenal-regeneration hypertension or reverses an established hypertension, affects primarily the fasciculata zone.[4] More recently, Dr. C. J. P. Giroud, of the Montreal Childrens' Hospital, has analyzed by bioassay adrenal effluent blood, which we have supplied from adrenal-regeneration hypertensive rats, for aldosterone content, with use of the method of Simpson and Tait.[13] Preliminary results suggest that the aldosterone secretion in

adrenal effluent blood of adrenal-enucleation hypertensive rats is not greater than that of normal rats of the same body weight. It must be concluded from these and other published data that the nature of the etiologic role of the adrenal cortex in adrenal-regeneration hypertension remains obscure.

## Summary and Conclusions

Hypertension with renal and cardiac hypertrophy and renal and vascular histopathological lesions has been produced in the rat coincident with delayed regeneration of enucleated adrenal glands. Animals in which compensatory adrenal hypertrophy occurred and animals in which the adrenals were either removed or left intact failed under the same conditions to develop hypertension. The results of these studies are interpreted as further evidence that as yet unknown factors related to the adrenal regeneration per se play a primary role in the development of hypertension during adrenal regeneration.

P. O. Box 6115.

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# The Histologic Patterns of Ruptured Myocardial Infarcts

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Rupture of the myocardium may be caused by trauma, infection, or neoplastic disease, but by far the commonest single cause is myocardial infarction due to arteriosclerosis of the coronary arteries. From a clinical point of view, every case of acute myocardial infarction is a potential case of rupture, since it is not possible to predict whether a particular patient will undergo rupture or even when rupture is most likely to occur. This unpredictability appears clearly in several series which show the variability of the interval between clinically apparent infarction and death due to rupture of the heart. An interval of 7 to 10 days was found most often by Edmondson and Hoxie.[1] More recent studies by Wesler, Zoll, and Schlesinger[2] and by Oblath, Levinson, and Griffith[3] show that rupture may occur at any time up to the fourth week, though principally (67%) in the first week and often (12%) in the first 24 hours. Intervals of as little as 10 to 12 hours are not rare, and in some cases both the presenting and the terminal symptoms have been those of rupture. The unpredictability arises from the fact that the mechanisms leading to rupture[4] are only partly understood, and so the significance of clinical events cannot be properly evaluated. This study of the histology of ruptured infarcts, based on material from the autopsy files of the Grace-New Haven Community Hospital from 1925 to 1954, was undertaken with the aim of determining the existence and nature of evidence for such mechanisms.

Attention has been drawn to certain general factors, which either increase the rupturing force (e. g., hypertension, lack of adequate bed rest) or decrease the ability of the ventricular wall to resist the force (e. g., age, general debility, myocardial atrophy). In this series (Table 1) only 60% of the patients were hypertensive (blood pressure greater than 160/100) before their infarcts, and in not all of that group did the hypertension persist or recur prior to rupture. Only two were receiving vasopressors at the time of rupture. The ages of the patients averaged 67 but ranged from 37 to 89. The gross specimens averaged 465 gm. in weight and 15 mm. in the thickness of the left ventricular wall. No predilection for a "weak spot" was found. There were two ruptures of the interventricular septum, one of the right ventricle and two of the posterior papillary muscle of the left ventricle. There were 33 perforations of the left ventricular wall, scattered over the anterior, lateral, and basal surfaces from base to apex. It seems clear that whatever may be the mechanisms of rupture, they can occur at any site in the heart and are only indirectly affected by systemic factors.

Two of the cases were deleted as not germane to the problem; one, a case of rupture of the interventricular septum with survival for almost a year, and the other, a case of dissecting hemorrhage from a fibrotic aneurism of the left ventricle. The remaining 36 cases were arranged in the order of the time interval between the clinical diagnosis of infarction and death

Submitted for publication April 6, 1956.

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TABLE 1.—*Myocardial Ruptures by Two-Year Periods from 1925 to 1954, with Total Number of Autopsies and of Deaths Due to Acute Myocardial Infarct without Ruptures*

| Years | Autopsies | Infarcts | | Ruptures | | Autopsies, % |
|---|---|---|---|---|---|---|
| | | No. | % | No. | % | |
| 1953–1954 | 1,136 | 104 | 9.1 | 13 | 12.5 | 1.14 |
| 1951–1952 | 857 | 52 | 6.1 | 3 | 5.8 | 0.33 |
| 1949–1950 | 724 | 35 | 4.8 | 2 | 5.7 | 0.40 |
| 1947–1948 | 810 | 71 | 8.8 | 5 | 7.0 | 0.62 |
| 1945–1946 | 736 | 38 | 5.2 | 5 | 13.2 | 0.67 |
| 1943–1944 | 678 | 35 | 5.2 | 3 | 8.6 | 0.44 |
| 1941–1942 | 797 | 33 | 4.1 | 2 | 6.1 | 0.25 |
| 1939–1940 | 792 | 32 | 4.0 | 0 | — | — |
| 1937–1938 | 563 | 28 | 5.0 | 1 | 3.6 | 0.18 |
| 1935–1936 | 586 | 17 | 2.9 | 1 | 5.9 | 0.17 |
| 1925–1934 | 2,532 | 30 | 1.5 | 3 | 7.7 | 0.12 |
| Total | 10,211 | 484 | 4.7 | 38 | 7.8 | 0.37 |

(Table 2). The chief difficulty in arranging this Table was the frequent uncertainty of clinical judgment in ascribing a time of onset to the presumptive infarct. Most cases presented a clear-cut incident, such as severe pain, shock, or collapse, which permitted the assignment of a definite time of onset of a major cardiovascular incident. However, in addition to the major incident there were usually multiple episodes of vague chest pain, dyspnea, "heart burn," weakness, nausea, "pneumonitis," etc. In two cases such "minor" incidents constituted the entire medical history, and in two others the patients had disclosed no premonitory symptoms of impending rupture. In view of the well-known occurrence of "painless infarcts," it is clear that the severity of symptoms may have little to do with the extent of the underlying damage. Hence, on clinical grounds it is reasonable to suspect that an event leading to rupture may or may not occur at the time of symptom formation or that a series of events is taking place prior to rupture, not all of which are symptomatic.

The peculiar incidence of rupture after infarction lends further support to this suspicion. From what is known of the histology of myocardial repair after infarction,[5] it would seem reasonable to expect rupture to occur most often between the 5th day, when dissolution of the necrotic fibers begins, and the 10th day, when the first visible fibrils of collagen appear. The actual incidence (Table 2) is maximal on the first and third days, remains somewhat elevated up to the seventh day, and thereafter drops sharply. It is difficult to accept a single infarct as the cause of rupture within 24 hours, as was seen in 10 cases, especially since no ruptures occurred in the second 24-hour period.

Fixed tissue was available from all of the specimens, but only seven of the gross specimens could be reexamined. In 14 of the remaining 29 it was fairly certain from the protocol or the tissue blocks that at least one section had been taken from the site of rupture. In 2 cases it was known that no sections were taken from the site of perforation, and in the other 13 it was not certain how close the blocks had been to the site. The sections were mounted in paraffin cut at 10$\mu$ and stained with hematoxylin and eosin or with Masson's trichrome.

## Observations

Histologically there were two or more stages of infarction in all but three of the

TABLE 2.—*Time Interval Between Clinical Diagnosis of Myocardial Infarction and Death Due to Rupture*

| Time | Males | Females | Total |
|---|---|---|---|
| Sudden | 0 | 2 | 2 |
| Up to 11 hr. | 0 | 0 | 0 |
| 11–24 hr. | 5 | 3 | 8 |
| 2d day | 0 | 0 | 0 |
| 3d-4th days | 5 | 5 | 10 |
| 5th-7th days | 5 | 3 | 8 |
| 2d wk. | 2 | 1 | 3 |
| 3d-4th wk. | 1 | 2 | 3 |
| †* | 2* | 0 | 2 |
| Total | 20 | 16 | 36 |

* In both of these cases there were multiple episodes of chest pain for several weeks, and it was not clear from these or from other evidence which was responsible for perforation.

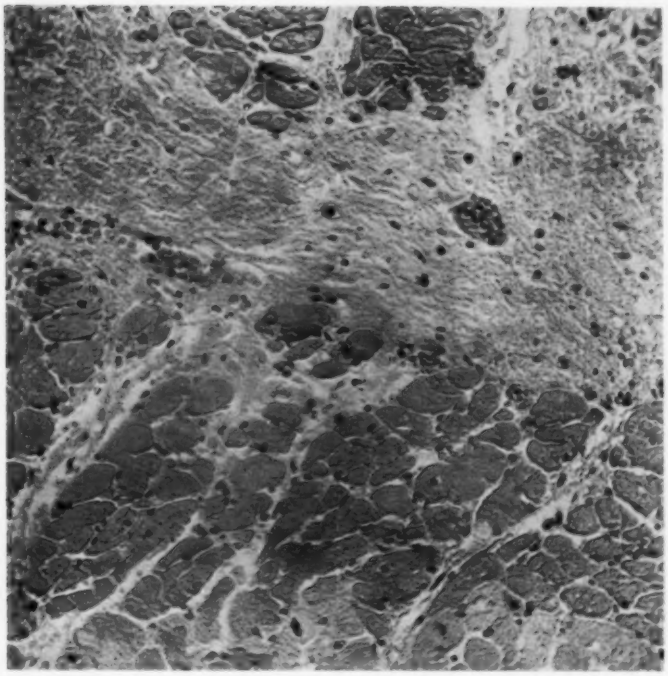

Fig. 1.—In the upper third of the field may be seen one of several small subendocardial scars. In the middle third may be seen a region of muscle fibers in a stage of moderately advanced necrosis which completely surrounded the scars. At the base may be seen fibers in an early stage of necrosis which were present across the remaining thickness of the ventricular wall. This field shows that the more advanced stage of infarction lay at the center of less advanced stages of necrosis, demonstrating a reversal of the expected "gradient of repair." Reduced about 10% from mag. × 256.

hearts. The more advanced stage or stages occupied the inner or subendocardial portion of the ventricular wall, while the less advanced lay in the outer portion of the wall, surrounding and including the more advanced stages. Such intermingling of stages has frequently been ascribed to a "gradient of repair" in a single infarct (e. g., Levine [5]). Such "gradients" for three reasons were thought to be incapable of accounting for most of the observed patterns: 1. In transmural sections the less advanced stages usually lay in the periphery of the infarcted region adjacent to the region of intact blood supply (Fig. 1), whereas in true "gradients" the more advanced stages lay peripherally. 2. The extent of the degenerative changes in the muscle fibers in the various stages was usually unequal, indicating that different regions had become necrotic at different times (Fig. 1). In true "gradients" the swelling, eosinophilia, and haziness of the necrotic muscle fibers were uniform; the "gradients" were seen in the cellular reaction to the infarcts. 3. In many of the older stages there was complete necrosis of the cellular infiltrate as well as of the muscle fibers, the only explanation being that the more advanced stages had also occurred earlier than the less advanced (Fig. 2). "Gradients of repair" noted in the largest of the older infarcts were readily identified by their unique admixture of cellular elements, with or without superimposed necrosis.

Within this histological pattern of superimposed older and younger infarcts there were wide variations, principally in the size and age of the older (smaller) infarcts. In most specimens the older stages appeared as multiple focal infarcts of fairly uniform size distributed through the inner ventricular wall, with sparing of the subendocardial layer of three to six fibers (Fig. 3) unless a mural thrombus was present. The larger the foci were, the more deeply did they extend toward the epicardial surface. In a few specimens the entire inner portion of the ventricular wall was infarcted with cellular infiltrates on both epicardial and

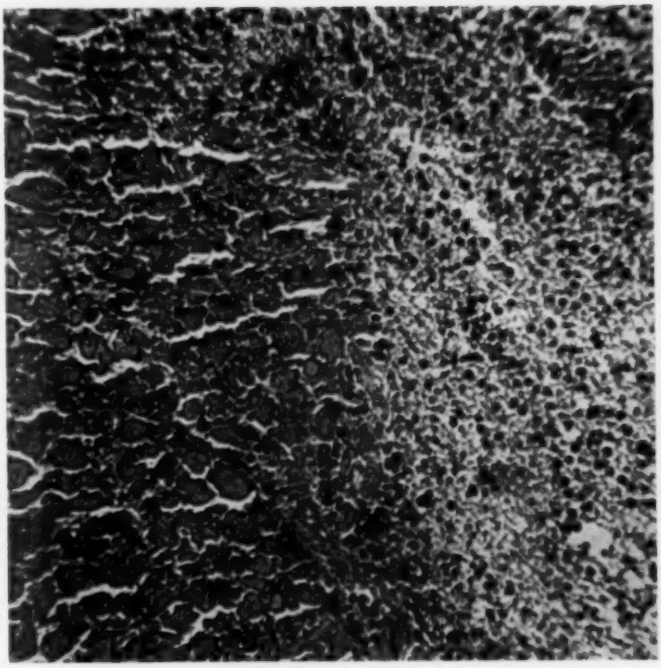

Fig. 2. — In the right half of the field may be seen a region of necrotic granulation tissue which adjoins a region of necrotic muscle fibers at the left. Both regions are surrounded by more recently infarcted tissue. The necrosis of the cellular reaction to necrosis is proof of the superimposition of infarcts, as opposed to the existence of "gradients of repair." Reduced about 10% from mag. × 256.

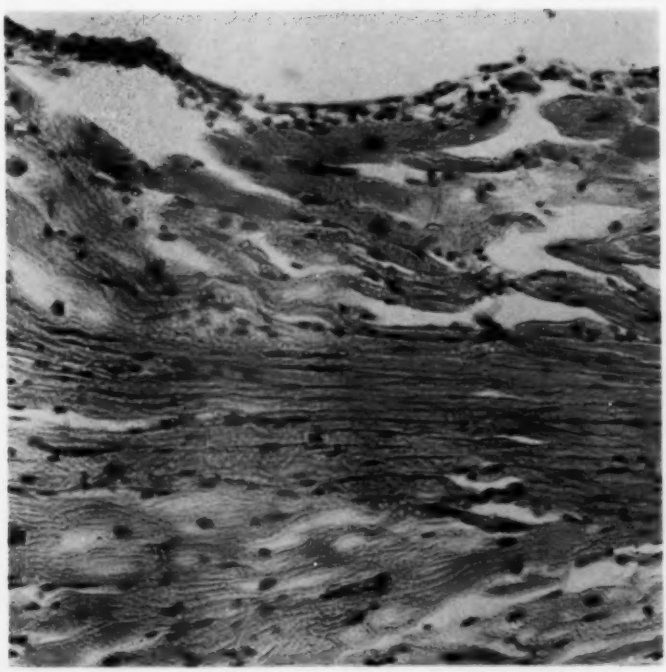

Fig. 3. — A trabecula carna is seen in longitudinal section. The subendocardial fibers do not show necrosis, but the fibers at the center show evidence of moderately advanced necrosis. In addition, the central fibers are abnormally thin, indicating that they have been stretched by a longitudinal force. Note that very few polymorphonuclear leukocytes are present. Reduced about 10% from mag. × 256.

endocardial margins but with a central zone devoid of leukocytes. Smaller infarcts of two, three, or more identifiable ages were often noted in such specimens within the subendocardial infarct. Despite this variety in appearance two elements were almost always present: 1. In all but four cases there were at least some regions (however small) in which eosinophilic degeneration of muscle fibers was well advanced but without dissolution of the fibers in association with macrophage infiltration. The exceptions were among those specimens from which the material for histological study was not known to have been taken from the site of rupture. 2. Transmural infarcts were present in all but one specimen. In seven hearts (including the two with ruptured papillary muscles) they were characterized by well-advanced necrosis with evidence of repair only at the periphery. In the other 28 cases they were very recent, with only slight to moderate eosinophilia of the fibers, swelling and lysis of the muscle nuclei, interstitial edema, and a sparse polymorphonuclear infiltrate along the periphery of the infarcts.

The gross specimens showed two types of perforation, which correlated fairly well with the two ages of the transmural infarcts. In the five cases with advanced transmural necrosis and in a few of those with early transmural necrosis, there was dilatation and thinning of the wall at the site of rupture, with gradual *rapprochement* of endocardium and epicardium. There was a single linear tear greater than 2 cm. in length, and the clotted blood in the pericardial sac showed no lamination. In 24 cases there was thinning only at the site of rupture, with a ragged abrupt margin suggesting hematoma formation in the wall prior to rupture. These specimens showed one or two short linear epicardial tears or occasionally only a pinpoint orifice, with no underlying clear-cut pathway into the ventricle. Microscopically at these sites there were great masses of red cells and fibrin extending tangentially into the wall between the muscle fibers. Radially oriented strands of fibrin were often seen, and occasionally there were great masses of thrombus in the wall, which could not readily be distin-

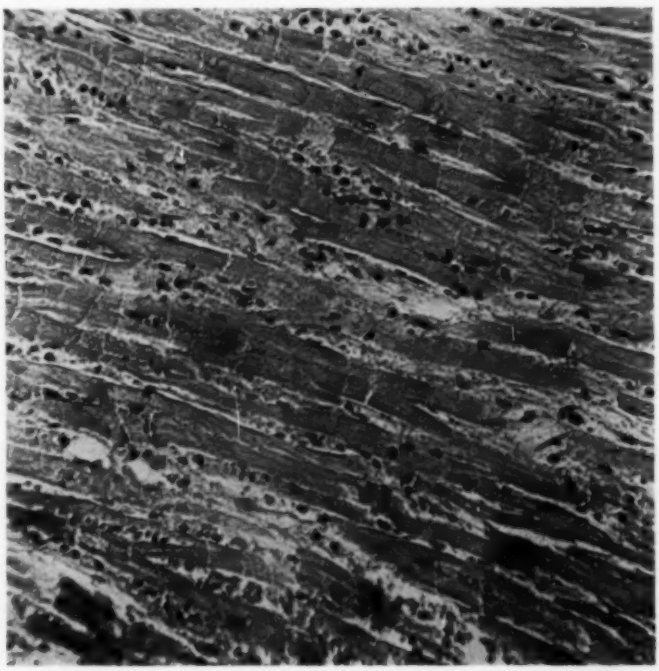

Fig. 4. — In contrast with Figure 3, which was taken at the same magnification, the necrotic fibers in the presence of a necrotic exudate show normal thickness but are ments, indicating a loss of cohesiveness and ductility. Reduced about 10% broken into short segfrom mag. × 256.

guished, if at all, from mural thrombus. Laminated clots were present also in the pericardial sac in six of these cases.

The effect of dilatation of the wall might reasonably be expected to appear as a thinning of the myocardial fibers in the stretched zone. This indeed was frequently seen, but characteristically this thinning occurred only in those fibers not surrounded by a necrotic exudate (Fig. 3). In regions where a necrotic exudate was present the fibers usually showed their normal diameter but in addition were broken up into short segments or else showed numerous transverse "fracture lines" crossing single fibers or small groups of fibers (Fig. 4). These did not correspond to intercalary disks and were frequently seen to lie at the sites of muscle nuclei. Although such "fracture lines" may well be artifacts incurred during the process of sectioning, they attest to the tendency of a necrotic fiber to break under tension rather than stretch when surrounded by a necrotic exudate.

The location of the transmural infarcts correlated well with the location of an occlusive coronary thrombus, which was found in all but four cases, one of the four being the case without a transmural infarct. Where the distance of the thrombus from the aorta was specified, it was less than 5 cm. in all but one case and less than 3 cm. in most. The thrombi appeared recent, i. e., they were composed of intact red cells and fibrin without organization at the periphery in all cases save two, in which one of two occlusions appeared older. These were the only known cases of multiple occlusion, although four of the histories had suggested much older infarcts and two of the specimens showed healed infarcts in sites other than those of perforation.

A search was made for occlusions that might account for the smaller, older infarcts. Three types were considered: multiple small arterial thrombi, distal thrombosis of a small artery with retrograde extension, and rupture of an arteriosclerotic plaque with embolization of the distal branches by the contents and subsequent thrombosis at the site of the plaque. No evidence of these processes was found. Considering the more or less coextensive distribution of the older and most recent infarcts, it then seemed most likely that a partial obstruction had occurred at the site of eventual thrombosis, sufficient to permit necrosis of a part of the muscle supplied by that vessel. The location of the smaller infarcts in the inner half of the wall was readily explained by the transventricular pressure gradient (during systole the arterial perfusion pressure is nil at the inner margin of the ventricular wall and equal to systemic pressure at the epicardial surface). Variation in size was explained by a variable degree and duration of ischemia and variation in age by recurrent bouts of ischemia. Three processes were considered: subintimal hemorrhage in a plaque with subsequent thrombosis, formation of a thrombus in layers with gradual occlusion of the orifice, and recurrent inflammation or edema of a plaque prior to occlusion with or without thrombosis. There was no evidence for the first or second possibilities; the third could not be directly proven with this material.

### Interpretation

Rupture of a heart results from intraventricular pressure and the tangential pull of myocardial fibers acting on a weakened zone in the wall. General factors play a role, such as age, sex, debility, hypertension, and lack of bed rest, resulting in a higher incidence of rupture in certain groups, but in this series these conditions were usually not present and were only rarely associated with minimal weight and thickness of the myocardium. A priori, one would expect maximal weakness to occur in a through-and-through infarct, the muscle fibers of which had been long necrotic and undergone minimal replacement by granulation tissue. These conditions were found in five of the ruptured left ventricles and in the two ruptured papillary muscles, comprising 20% of the series. Such infarcts are relatively

uncommon, and their high incidence here suggests that they are more prone to rupture. But the majority of hearts, almost 70%, did not show this; rather there was focal advanced necrosis in the inner part of the wall with surrounding quite recent necrosis extending to the epicardium. The extent and degree of these changes were sometimes so slight as to be very puzzling when comparison was made with other hearts with severe infarcts which had not ruptured prior to death. Even more than late transmural necrosis, this focal-general pattern appeared to constitute the anatomical substrate of weakness.

How can such a pattern lead to weakness? One must consider the effect of the more recent infarct superimposed on the three elements of the older, i. e., on the vessels, the cellular infiltrate, and the remaining fibers.

First, the occurrence of intramural hemorrhage from necrotic arteries (which were frequently seen) cannot be denied, but its significance is made questionable by the fact that only 4 of these cases out of 28 in the last eight years of the series were receiving anticoagulant therapy at the time of rupture, despite extensive clinical use of the anticoagulant drugs.

Second, if there was sufficient blood supply to enable myocardial fibers to survive between focal infarcts, even as deep as those juxtaposed to fibers supplied from the ventricle, then there must have been enough to bring in the polymorphonuclear infiltrate so characteristic of these foci. Were this not so it would be difficult to explain how leukocytes could penetrate so deeply into the necrotic tissue, since their limits of penetration were clearly limited in those specimens with necrosis of the entire inner half of the infarcted wall. Transmural necrosis would kill this infiltrate as well as the remaining fibers. Such massive necrosis is not usually seen in the ordinary evolution of an infarct but constitutes a striking feature of many of these specimens. Evidence for a weakening action of a necrotic exudate on the muscle fibers was found in the remarkable difference between those fibers stretched in its presence (Fig. 4) and in its absence (Fig. 3). According to the studies of Mallory, White, and Salcedo-Salgar,[6] the degree of a polymorphonuclear exudate increases over the first 4 days, with the earliest onset of degenerative changes in the cells at about 48 hours. By the fifth and sixth days their number stabilizes as the influx stops. During the 2d week their number diminishes rapidly, until by the 14th day they have practically disappeared. In referring to Table 2, it is notable that the maximal incidence of rupture coincides with the maximal infiltration of polymorphonuclear leukocytes. The correlation indicates that the necrotic exudate in focal infarcts accounts in part for the weakness associated with the focal-general pattern.

Third, in a number of cases the polymorphonuclear exudate was quite sparse. Although one can surmise that it may have been dense in the region destroyed by hematoma, it seems wiser to introduce another factor, the effect of ischemia on the remaining viable muscle fibers. Rigor mortis is known to occur in cardiac muscle,[7] and it is reasonable to suppose that its in vivo counterpart, ischemic contracture, occurs also. This phenomenon may enter in two ways. Focal infarcts destroy the continuity of the myocardium, leaving intervening and surrounding muscle fibers free to retract out of the infarcted zones. Formation of a transmural infarct surrounding the smaller zones would convert an intermittent contraction to a steady traction, which would facilitate the formation of a defect in the inner portion of the ventricular wall and predispose to hematoma. After onset of the transmural infarct the outer layer of muscle would form a rigid splint, at once containing the defect or hematoma and acting as an anchor for the rest of the heart to pull against. But as rigor wore off, this containing action would disappear, with an outcome either of acute left ventricular failure or of rupture of a preformed hematoma.

The loss of the splinting effect of ischemic contracture would be most likely to be disastrous in a large infarct. In accordance with this the thrombi found in these cases were located in the proximal portion of a major vessel. If ischemia is severe enough, thrombosis need not occur at all, as was apparently the case in four of these specimens.

The inference arises from this analysis that coronary thrombosis is not an "all-then-nothing" process but is preceded by recurrent periods of partial obstruction of variable duration and progressive severity. In the absence of more direct evidence of other events, this ischemia seems most likely due to recurrent edema in an arteriosclerotic plaque.[8] Infarction occurs prior to thrombus formation, and in at least two out of three of the patients in this series such "preliminary infarcts" appeared to give rise to the severest symptoms. The time of formation of a thrombus could not be specified from examination of the clot itself, but if it is accepted that the thrombi found were the cause of the transmural infarcts found, then it appears that thrombus formation may closely precede death without producing symptoms in excess of what has gone before. Rupture appears most likely to occur when (1) ischemia due to partial obstruction of a major artery has caused focal necrosis in the inner portion of the ventricular wall; (2) thrombosis of the artery at the site of partial obstruction has followed the ischemic necrosis by three to seven days, when necrosis is advanced and a maximal polymorphonuclear infiltrate is present; (3) ischemic contracture of the ventricular wall has abated. It may also occur through a transmural infarct more than a few days old, in which the process of repair has not proceeded rapidly enough to support the deteriorating necrotic muscle.

## Summary

In 36 cases of death caused by rupture of the myocardium after infarction due to arteriosclerosis, the commonest intervals between clinically apparent infarction and death were less than 24 hours and from 3 to 7 days. Histological examination of the hearts demonstrated multiple superimposed infarcts, the commonest pattern being that of multiple subendocardial infarcts of an indicated age of three to seven days, surrounded by a very recent transmural infarct, associated with a single occlusive thrombus in a major coronary artery. Indirect evidence indicated that the older, smaller infarcts were caused by partial obstruction of the major vessel at the site of eventual thrombosis. The hypothesis is proposed that rupture is most likely to occur when ischemic infarcts are followed in three to seven days by coronary thrombosis and a superimposed transmural infarct.

Department of Physiology, University of California Medical Center (24).

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# Experimental Membranous and Nodular Glomerulosclerosis

*Induction by "Fibrinoid" Derived from Autolyzed Smooth Muscle*

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Fibrinoid may be encountered within connective tissue and within small arteries and arterioles.[1] The derivation of fibrinoid as encountered within the wall of small arteries and arterioles from damaged and disintegrated vascular smooth muscle has been proposed.[1-11] In certain states[2,8,9,11] not only is the fibrinoid observed within the wall of muscular vessels but it may be found in the lumen of such vessels and within the lumen of nearby capillaries, where smooth muscle is normally absent. An attempt has been made to interpret the presence of certain forms of fibrinoid within capillaries by proposing an embolic origin.[2,8,9,11] The latter view considers that "vascular fibrinoid" [1] found within arteries and arterioles may swell and spread centripetally into the lumen, where it may be dislodged, be swept along the blood stream, and embolize nearby capillaries.

Fibrinoid of the "vascular type" [1] may be encountered within small renal arteries and arterioles, glomeruli, Bowman's capsule, renal tubules, and the peritubular areas in such varied states as diabetes mellitus,[9] malignant or accelerated hypertension,[10] and the Shwartzman reaction.[8] Clearly in these sites the fibrinoid must either be brought there as such by body fluids or it must be developed locally from ingredients which may be contributed to by the body fluids. In considering this unique distribution of fibrinoid in these conditions we have suggested an origin from the smooth muscle of renal arteries and arterioles followed by a migration to the other sites. Collateral support for this interpretation was developed by the demonstration that particles of autolyzed normal smooth muscle have tinctorial and histochemical properties which are similar to those of fibrinoid as encountered in the wall of arteries and arterioles and in the glomeruli and tubules in disease states. Disintegrated normal smooth muscle resembles "vascular fibrinoid" not only in the test tube but also in the kidney after its injection into the renal artery.[10] Moreover, particles of autolyzed smooth muscle have been demonstrated to rupture through the glomerular capillaries and lodge in the capsular space and within tubules as casts.

In malignant or accelerated hypertension and in diabetes mellitus not only is fibrinoid of the "vascular type" observed within the glomeruli but the glomeruli undergo morphologic alterations which have been designated as "alterative glomerulitis" [12] and "glomerulosclerosis." [13,14] It was only natural to raise the question concerning a possible relationship between the fibrinoid present within glomeruli and the additional glomerular alterations.[9]

In view of these considerations it was decided to evaluate the influence of particles of autolyzed smooth muscle lodged in the glomerulus for a few weeks on the morphologic appearance of the glomerulus and its capsule.

Submitted for publication Oct. 28, 1957.

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Supported by a grant from the National Heart Institute, National Institutes of Health, U. S. Public Health Service.

## Material and Methods

The autolyzed smooth muscle was obtained and prepared from normal dogs as previously described.[10] In preparing the muscle approximately 50 to 75 gm. of muscle obtained under sterile operating-room conditions was suspended in 500 ml. of M/10 phosphate buffer (pH 7.4) and subjected to the action of a Waring Blendor. The ultimate particle size was obtained by passage through sieves of diminishing porosity. The quantity of muscle in the final preparation was not known. By experience it was determined that a volume of 30 to 50 ml. of the final suspension yielded a considerable degree of glomerular embolization, with minimal and frequently no gross renal infarction.

The suspension was cultured after its preparation and in the course of the experiments. The routine bacterial cultures yielded no growth.

Healthy mongrel dogs, weighing 7 to 15 kg., were subjected to thiopental (Pentothal) anesthesia. The abdomen was shaved and prepared with antiseptic soap and a benzalkonium (Zephiran) solution. Through a midline incision one kidney was removed after the pedicle had been clamped and ligated. This kidney became the control kidney. The renal artery and vein of the remaining kidney were dissected and isolated. A bulldog clamp was placed across both artery and vein behind the bifurcation of the artery. Through a 22 gauge needle 30 to 50 ml. (in one case 70 ml.) of the suspension of autolyzed muscle was injected into the renal artery toward the kidney. After the injection the clamp was removed from the renal artery but left on the renal vein. After 30 to 60 seconds the clamp on the renal vein was removed. Hemostasis was secured by gentle pressure on the renal artery. The abdomen was closed in layers.

In one instance an alternative procedure was used. The aorta below the renal arteries was clamped. The renal veins were isolated and clamped. Then 30 ml. of the suspension of muscle was injected into the aorta above the renal arteries. The clamps on the aorta and renal veins were allowed in place for three minutes. There was no control kidney; otherwise the procedure was equally satisfactory.

The dogs were killed and the remaining kidneys were removed at intervals varying between 6 and 56 days (Table 1). The results derived from eight such experiments are being reported.

The kidneys (control and test specimens) were fixed in 10% buffered formalin. Random sections were prepared. The following stains and histochemical procedures according to the references previously given[10] were used for each test specimen and its control: hematoxylin and eosin, phosphotungstic acid hematoxylin (PTAH), Masson trichrome, Mallory aniline blue, Wilder's silver, the periodic acid-Schiff reagent (PAS), and oil red O.

TABLE 1.—*Data on Experiments on Eight Dogs*

| Dog | Smooth-Muscle Suspension Injected, Ml. * | Dog Killed, Days After Injection |
|---|---|---|
| 1 | 50 | 6 |
| 2 | 50 | 10 |
| 3 | 30 | 13 |
| 4 | 30 | 15 |
| 5 | 30 | 23 |
| 6 | 40 | 29 |
| 7 | 40 | 30 |
| 8 | 70 | 56 |

* Volume of smooth-muscle suspension injected into the renal artery for each dog.

## Results

While the control kidneys were normal in appearance both grossly and microscop-

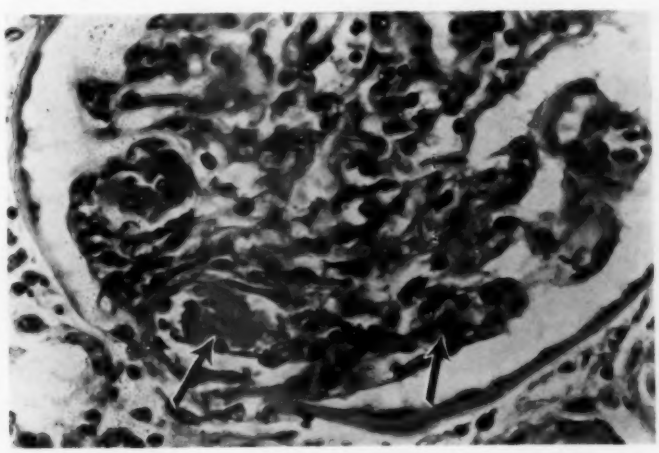

Fig. 1 (Dog 1).—At the arrow on the left side and above and to the left of it two masses of "fibrinoid" within capillary loops are displayed. About these there is cellular proliferation. These cells appear to be of endothelial origin. At the arrow on the right there is a mitotic figure. Otherwise generalized increased cellularity and some degree of membranous thickening are present. Hematoxylin and eosin; reduced 40% from mag. × 720.

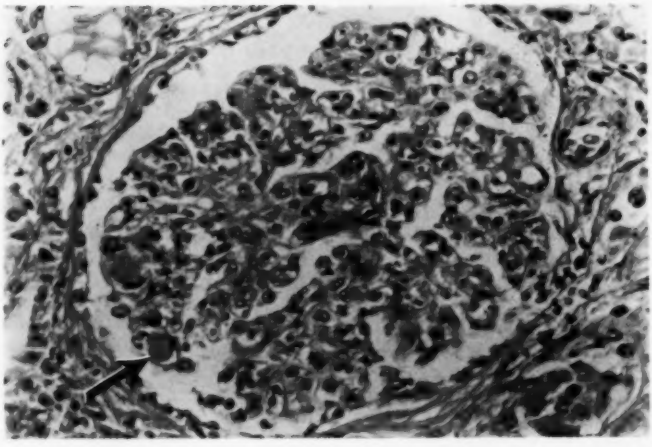

Fig. 2 (Dog 1).—Generalized hypercellularity is shown. A globule of "fibrinoid" is present at arrow and above it. These masses appear to be within capillaries. Some degree of intercellular thickening is present, but this is finely granular and not hyaline as in other examples. Hematoxylin and eosin; reduced 40% from mag. × 515.

ically, the kidneys which received injections revealed major changes, especially within the glomeruli. The changes were particularly evident when tissue blocks were serially sectioned.

*A. Glomerular Changes.*—1. "Fibrinoid" Deposits: Deposits of "fibrinoid" were present within glomerular capillaries (Figs. 1 and 2). In some glomeruli the fibrinoid was lumpy and partly granular, while in others it assumed a smooth hyaline appearance. Up to eight weeks after the injection the fibrinoid deposits in the glomeruli revealed the same tinctorial and histochemical characteristics as autolyzed smooth muscle in the test tube and within the kidney shortly after its injection.[10]

2. Cellularity: Proliferation of fixed cells was evident about the fibrinoid deposits (Fig. 1). These cells appeared to be endothelial cells. A rare mitotic figure was seen in the glomerulus near the "fibrinoid" deposits (Fig. 1).

Diffuse hypercellularity was also noted (Figs. 1, 2, and 3). Between the cells there was an increment of intercellular material which resembled basement membrane. These changes were either widespread over the glomerulus or confined to a lobule. The cellularity appeared to be mainly near the lumen of the capillaries, which were at the same time reduced in caliber. In some instances the capillary wall was thickened by a concentric accumulation of cells (Fig. 4).

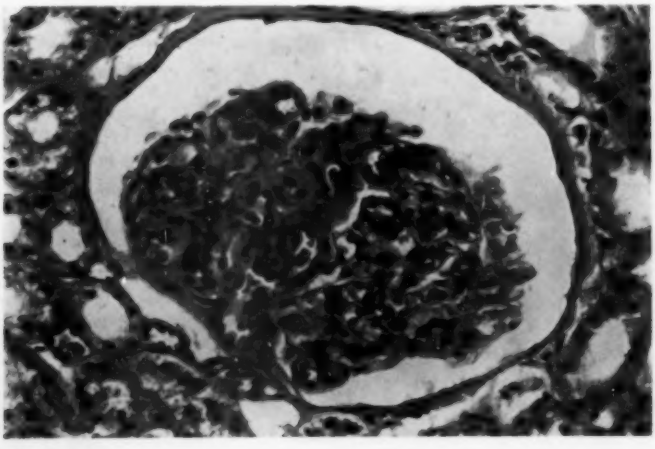

Fig. 3 (Dog 4).—The left half of this glomerulus is mostly destroyed by increased cellularity and intercellular hyaline thickening. The cells appear to be mostly of endothelial origin. The right half of the structure is reasonably preserved. Bowman's capsule is thickened. Hematoxylin and eosin; reduced 40% from mag. × 515.

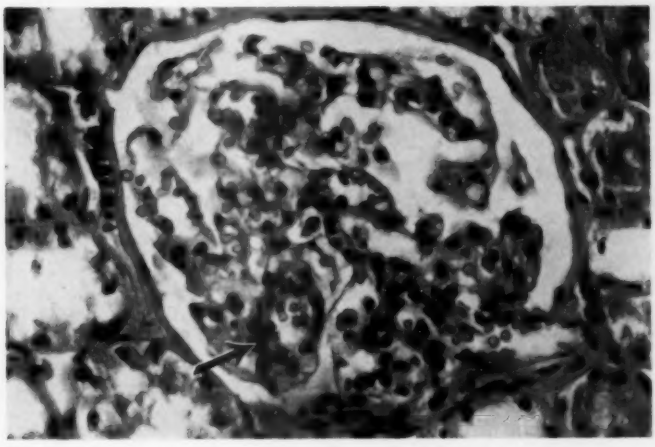

Fig. 4 (Dog 4).—Bowman's capsule reveals hyaline thickening. At the arrow a capillary loop demonstrates a thickened wall due to the concentric proliferation of cells which resemble endothelial cells. Another similar lesion is present to the right of this one. Hematoxylin and eosin; reduced 40% from mag. × 720.

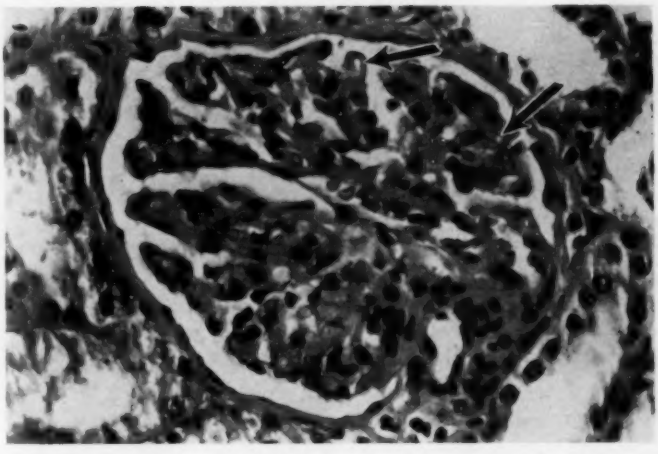

Fig. 5 (Dog 4).—A glomerulus 15 days after the injection. The main changes consist of capillary (or intercapillary) thickening of the diffuse membranous type. At the sites of the arrows the changes resemble wire-loop configuration. Bowman's capsule is also thickened. The hilar cellularity is not considered significant; however, the hilar hyalinization may be significant. Hematoxylin and eosin; reduced 40% from mag. × 845.

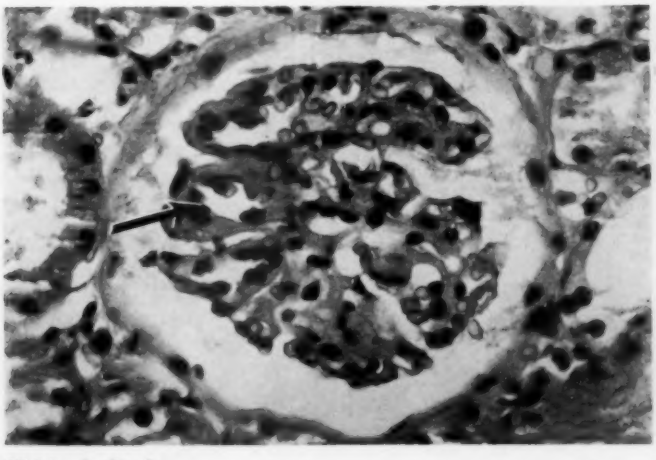

Fig. 6 (Dog 4).—This photomicrograph shows the diffuse membranous change. The wire-loop change is suggested. At the arrow the alteration of the glomerular membrane is brought into focus as the endothelium, thickened area of the basement membranes, and an epithelial cell can be made out. An increase in the endothelial cells appears present in some capillary loops. Bowman's capsule is thickened, but the material here is more granular than hyaline. Hematoxylin and eosin; reduced 40% from mag. × 860.

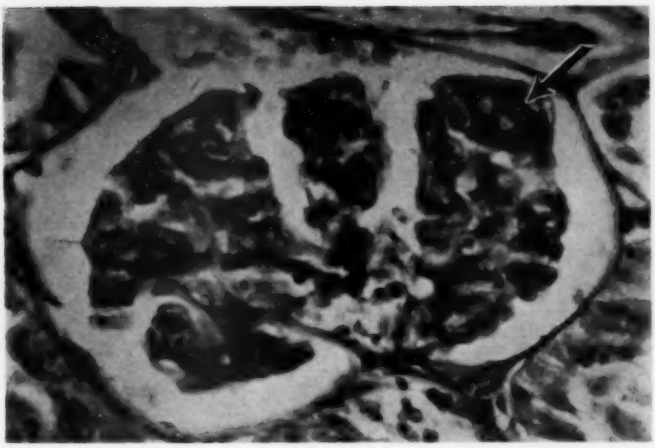

Fig. 7 (Dog 7).—The PAS-positive nature of the diffuse membranous lesion is shown. A wire-loop lesion is shown at the arrow. The PAS-positive material has thickened the capillary wall and encroached on the lumen of this vessel. Periodic acid Schiff; reduced 40% from mag. × 740.

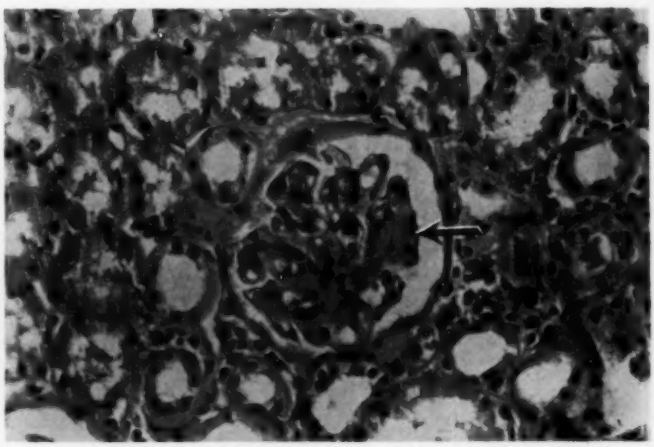

Fig. 8 (Dog 4).—This glomerulus is approaching destruction by sclerosis. The sclerosis appears due mainly to a diffuse membranous thickening. A nodular configuration is present at the arrow. Elsewhere there are wire-loop-like changes. Hematoxylin and eosin; reduced 40% from mag. × 515.

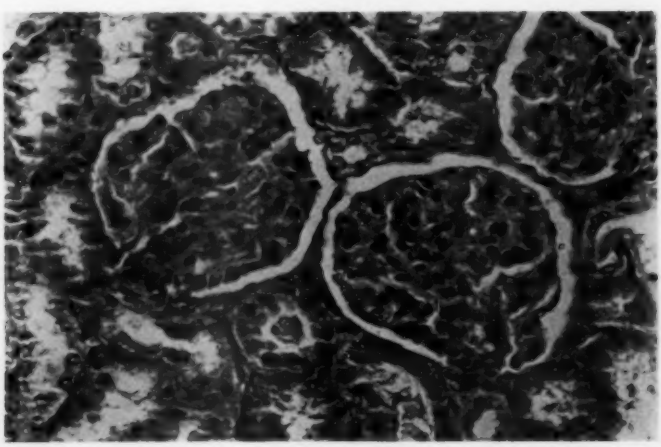

Fig. 9 (Dog 2).—Two glomeruli showing the diffuse capillary or intercapillary lesion. Such structures reveal the appearance of decreased vascularity. Hematoxylin and eosin; reduced 40% from mag. × 515.

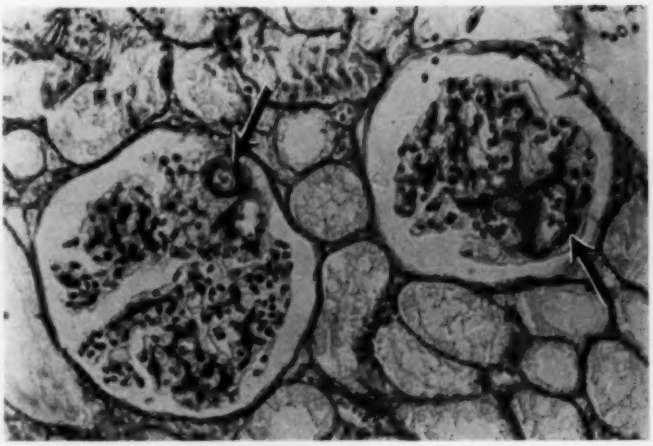

Fig. 10 (Dog 4).—Silver-positive material in the wall of thickened capillary is shown. The black fibrils are present in the hyaline and membranous foci, as shown by the arrows. Bowman's capsule also contains black fibrils. Silver; reduced 40% from mag. × 460.

The impression was gained that these cells were of endothelial origin.

3. Membranous Changes: The capillary walls were thickened in a manner resembling "diffuse intercapillary sclerosis" and the "wire-loop" lesion (Figs. 5 to 10). This change resulted primarily from a hyaline thickening apparently involving the capillary wall, to which the term membranous thickening may be applied. The capillary lumina were frequently narrowed by the thickening of the capillary walls. In some glomeruli or within lobules of a given glomerulus diffuse thickening with choking destruction occurred (Figs. 3, 5, and 8.). More often the glomerular capillaries were open, the endothelium was identified, but the capillary wall was thickened giving the "wire-loop" configuration( Figs. 6, 7, and 10). Occasionally an entire lobule or glomerulus was sclerosed and apparently destroyed by this process( Figs. 3, 8, and 9).

4. Capsular Changes: The capsular space was usually empty, but in some instances it contained a proteinaceous precipitate (Figs. 6 and 12). The parietal layer of Bowman's capsule was frequently thickened (Figs. 1, 3, 4, 6, 8, 10, and 13). This was usually a hyaline thickening (Figs. 1, 4, 5, and 13), although in some examples the material in the thickened capsule was finely granular (Figs. 6 and 8). The capsular thickening was usually diffuse over the capsule, but on occasions it was localized,

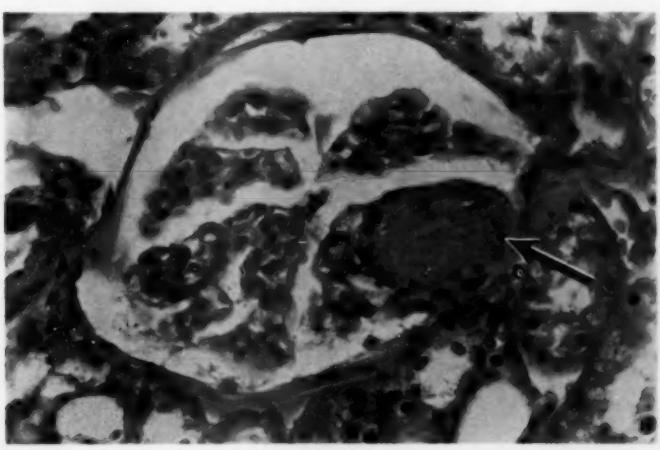

Fig. 11 (Dog 4).—The nodular structure shown has a central core and peripheral cells which are within the structure of the nodule. To one side of the nodule are open capillaries. The capsular space contains a protein precipitate. Hematoxylin and eosin; reduced 40% from mag. × 630.

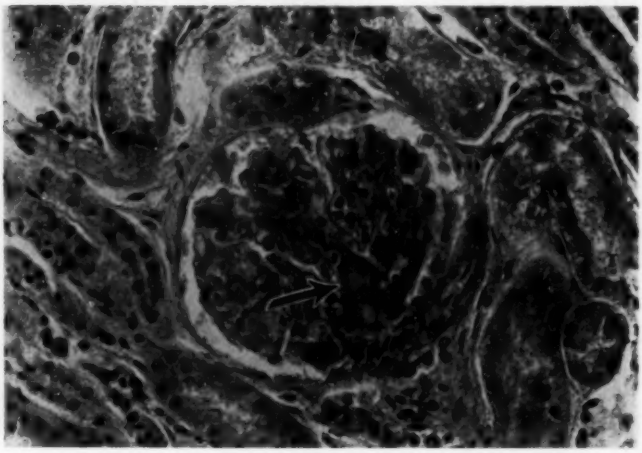

Fig. 12 (Dog 7.)—A nodular structure in the central axis of the glomerulus not far from its hilus is shown. The remainder of the glomerulus is either partly collapsed or the seat of increased cellularity. Hematoxylin and eosin; reduced 40% from mag. × 515.

as in Figures 8 and 13. The cells in the capsule were applied to the inner side as in the normal state. Occasionally the nuclei of of the capsule appeared larger than normal.

The hyaline material of the capsule had the same tinctorial characteristics as the glomerular capillary (or intercapillary) thickening. These materials were eosinophilic and stained blue with Mallory's aniline blue, green with Masson's trichrome stain, orange-brown with phosphotungstic acid hematoxylin, and purple with the PAS procedure. With Wilder's silver stain the material was brown, but in addition it had black-silver-positive fibrils. This material was not sudanophilic (Table 2).

5. Nodular Lesion: The lesions considered as nodular are depicted in Figures 11, 12, and 13. These structures had a central core surrounded by cells with dark flattened or oval nuclei. An occasional cell appeared to be within the core. The core usually was hyaline in appearance, although in some instances there was in addition a fine crinkled or granular appearance.

The core of the nodule displayed tinctorial characteristics similar to those of the thickened capillary wall and Bowman's capsule. This included the presence of silver-positive material.

*B. Vascular Changes.*—The fibrinoid change was observed within the wall of

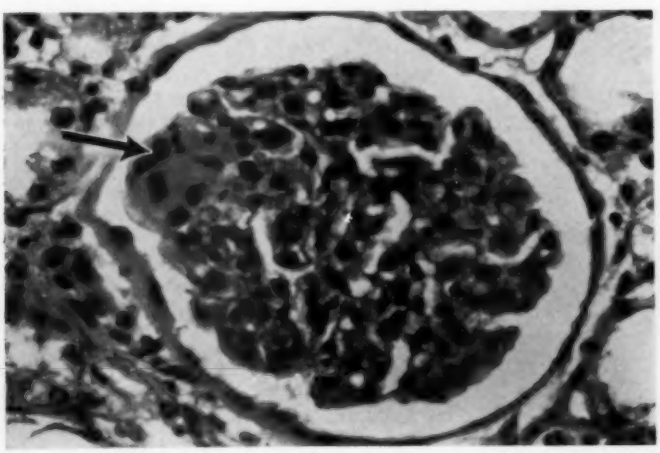

Fig. 13 (Dog 4).—The central core of the nodular structure shown has a hyaline appearance. Nearby Bowman's capsule is thickened. Hematoxylin and eosin; reduced 40% from mag. × 880.

TABLE 2.—*Tinctorial Characteristics of Fibrinoid and Glomerular Lesions* *

| Procedure | Fibrinoid in Artery or Glomerulus | Membranous or Capillary Thickening (Wire Loop) | Nodule | Bowman's Capsule |
|---|---|---|---|---|
| Hematoxylin and eosin | Eosinophilic | Eosinophilic | Eosinophilic | Eosinophilic |
| Mallory aniline blue | Red (some blue admixture) | Blue | Blue | Blue |
| Masson's trichrome | Red (some green admixture) | Green | Green | Green |
| PTAH | Orange-brown & purple | Orange-brown | Orange-brown | Orange-brown |
| Wilder's silver | Brown (neg.) | Brown plus black fibrils (+) | Brown plus black fibrils (+) | Brown plus black fibrils (+) |
| PAS | Purple (+) | Purple (+) | Purple (+) | Purple (+) |
| Oil red O | Red (+) | Negative | Negative | Negative |

* The capillary ("intercapillary" or "wire-loop" lesion), the nodules, and the parietal layer of Bowman's capsule gave similar results, and these differed from those of the fibrinoid (injected material).

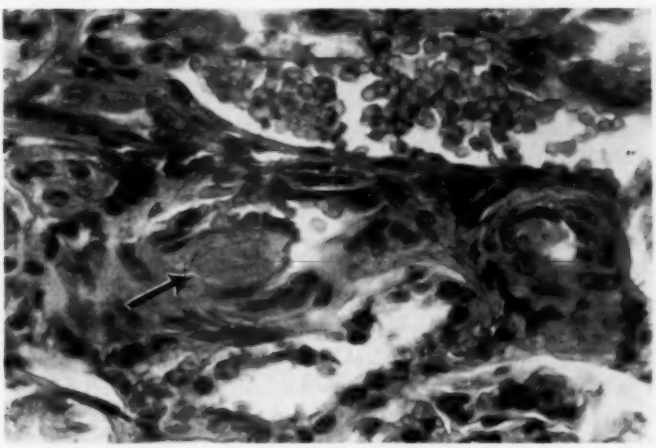

Fig. 14 (Dog 6).—The polypoid mass of "fibrinoid" covered by endothelium within a small artery was observed 29 days after injection. Hematoxylin and eosin; reduced 40% from mag. × 850.

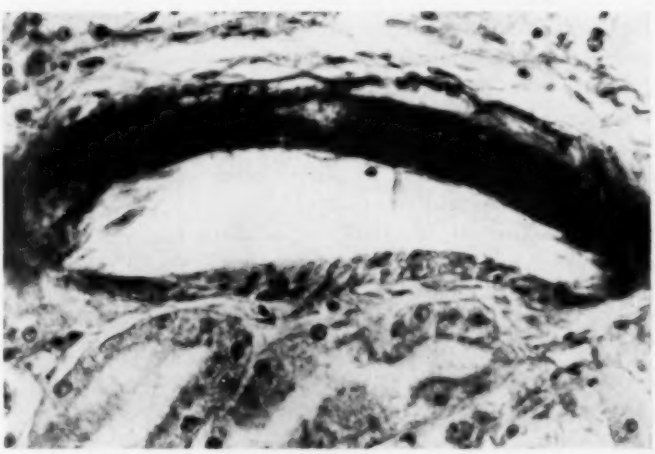

Fig. 15 (Dog 4).—An artery showing replacement of a segment of the wall by PAS-positive material. This area is in direct continuity with intact media. The general appearance of this and other vessels suggests a blending of injected "fibrinoid" and "fibrinoid" derived locally from the vessel wall. Periodic acid Schiff; reduced 40% from mag. × 700.

interlobular arteries as well as within their lumina. In interlobular arteries the fibrinoid was frequently attached to the wall of the vessel and covered by endothelium (Fig. 14). It either formed a small deposit on the intima or projected into the lumen in the shape of a sessile polyp. Where the deposit was small the wall of the vessel appeared intact and the fibrinoid appeared to be part of the intima. Where the deposit was larger the fibrinoid involved a portion or all of the vessel wall (Fig. 15).

The fibrinoid in the interlobular arteries had the same tinctorial and histochemical characteristics as autolyzed smooth muscle in the test tube and vascular fibrinoid in small arteries and arterioles. The finding of fibrinoid two to eight weeks after the injection with the same characteristics as shortly after the injection suggests that the material is often inert and stable and by being covered by endothelium may become incorporated into the vessel wall. Occasionally the entire arterial wall demonstrated the fibrinoid changes, and the impression was gained that the injected smooth muscle ("fibrinoid") became meshed with the injured smooth muscle of the arterial wall ("locally developed fibrinoid"). The latter possibility is demonstrated in Figure 15.

*C. Tubular Changes.*—Focal degeneration and necrosis of the tubules was noted in some sections. These degenerative changes were separate and distinct from infarcts. Infarcts were uncommon in the present experiment, presumably because the particles injected were very small. Within these damaged tubules as well as within other tubules which appeared intact casts of "fibrinoid" were observed. The ability of particles of autolyzed smooth muscle to pass through the glomerular capillaries and lodge in tubules had been previously demonstrated. The present observations suggest that such casts may remain for a considerable period of time (two to eight weeks) or re-form periodically.

## Comment

The fibrinoid material within the interlobular arteries and glomeruli in the present experiments displayed the same appearance and characteristics one to eight weeks after the injection as it did in the test tube and within the kidney shortly after its injection into the renal artery in former experiments.[10] It is reasonable to consider the intraarterial and intraglomerular fibrinoid observed after one to eight weeks as the material injected. The injected material in turn had its origin from normal smooth muscle.

The injected fibrinoid in most instances appeared to be inert insofar as the classical inflammatory response was concerned. It is true that an occasional artery containing this material revealed a damaged wall which was infiltrated with leukocytes and some involved glomeruli contained scattered neutrophilic leukocytes, but in the main, inflammatory cells were not associated with the lodging of this material. Moreover, this fibrinoid material did not evoke classic thrombosis in the sense of the deposition of platelet columns with marginal leukocytes, fibrin strands, and enmeshed erythrocytes. Instead within arteries the fibrinoid became covered by endothelium and at least in some instances appeared to remain unaltered for as long as eight weeks. Within the glomeruli some fragments of fibrinoid remained intact, although here a proliferation of cells resembling endothelial cells was evoked.

It would seem that one of the main reactions to the intravascular lodging of the fibrinoid consisted of the proliferation of endothelial cells. This observation is in keeping with observations related to the vascular fibrinoid of small arteries after bilateral nephrectomy of the dog.[11] The mural fibrinoid following renal ablation, which has been considered as derived from the medial smooth muscle, may swell into the lumen and form a polypoid mass. This mass not only becomes covered by endothelium, but it may be invaded and organized by cells which resemble endothelial

cells. To what extent fragments of fibrinoid (injected or derived in vivo) disintegrate into the circulating stream has not been ascertained.

The demonstration of a relative inertness and stability of certain of the fragments of injected fibrinoid can be considered to support the thesis which considers altered vascular smooth muscle as the basic source of vascular fibrinoid and vascular hyalin. There is, by virtue of the present observations, experimental evidence supporting the ability of disintegrated smooth muscle to remain in situ for prolonged intervals without undergoing total dissolution, without evoking a major inflammatory response, and without initiating classical thrombosis.

Migration of the fibrinoid plus its subsequent lodging and covering by endothelium may explain the occasional encounter of a globule of subendothelial fibrinoid or hyalin. The incorporation of hyalin into the arteriolar wall by such mechanism has been proposed by Duguid and Anderson.[15] The presently considered views differ from those of the latter workers in that the basic source of arteriolar fibrinoid or hyalin is considered to be altered smooth muscle.

The main lesions observed in the present study were glomerular. These lesions basically emphasized cellular proliferation and membranous and nodular changes. The increased cellularity seemed to involve endothelial cells when it occurred about a deposit of fibrinoid. When the capillary wall was thickened due mainly to an increased cellularity the cells resembled endothelial cells. Where diffuse cellularity pertained one could not be certain whether cells other than those of endothelial origin had proliferated. Cells were also present in and about the nodular structures.

The membranous changes consisted basically of a hyaline thickening about the endothelial lining of the capillaries, as described by Allen [16] for human disease. The area of involvement consisted of the general area of the basement membranes, but whether the material was identical to that of the normal basement membranes and whether the endothelial or epithelial membranes or both membranes were involved could not be ascertained with the use of the light microscope alone. A similar hyaline thickening involved the area of the epithelial basement membrane of the parietal layer of Bowman's capsule. The nodular changes were not as common as the cellular and membranous changes.

The intercellular material, the membranous substance, the matrix of the nodules, and the capsular hyalin displayed tinctorial properties which differed significantly from those of the fibrinoid material. Thus, the experiments indicate the stimulation of the deposition of new material in the form of the membranous, nodular, and capsular deposits. These changes may be considered as due to reaction to injury by the glomerulus. According to the setting of these experiments, the injury was initiated by a form of embolization. The observations, however, suggest that the injury was not due solely to embolization but may have resulted from products from the fibrinoid (autolyzed smooth muscle), which stimulated cells within the glomeruli not only to proliferate but also to deposit the membranous (capillary), nodular, and capsular material. The latter interpretation appears to be supported by the presence of the changes in many glomeruli not containing fibrinoid and the diffuse changes in Bowman's capsule away from the sites of lodging of the emboli.

## Summary

Autolyzed normal smooth muscle made finely particulate was injected into the renal artery of the dog, and the kidney so disturbed was studied morphologically one to eight weeks later. After this interval fibrinoid was observed within interlobular arteries and glomeruli. This fibrinoid displayed the same characteristics as the autolyzed smooth muscle in the test tube and the autolyzed muscle shortly after its injection into the kidney. The fibrinoid observed in the kidney one to eight weeks after the injection appeared to be the injected material

prepared in the test tube from smooth muscle.

One to eight weeks after the injection of fibrinoid derived from normal smooth muscle major morphologic changes transpire within the glomeruli. These glomerular lesions emphasize increased cellularity, both diffuse and about fibrinoid deposits in the glomeruli; capillary or intercapillary membranous thickening resembling the diffuse membranous alteration and the wire-loop lesion; nodular lesions having a central eosinophilic core and peripheral cells, and hyaline thickening of the parietal layer of Bowman's capsule.

The cellularity and membranous thickening within the glomeruli give rise to varying degrees of glomerular destruction by sclerosing this structure.

In considering the fibrinoid material as the injected particles, it would seem that this type of fibrinoid may remain up to eight weeks within small arteries without evoking a major inflammatory response while maintaining its tinctorial properties. Within the arteries this fibrinoid becomes covered by endothelium, while within the glomerulus it stimulates the proliferation of cells about it. The latter cells appear to be of endothelial origin.

Where the fibrinoid is pressed against the intima the entire thickness of the vessel may assume the fibrionid appearance. This finding has suggested damage to the local smooth muscle and fusion of fibrinoid derived locally from vascular smooth muscle and the injected fibrinoid derived from extravascular normal smooth muscle.

The tinctorial properties of the membranous thickening, the nodular lesions, and the thickening of Bowman's capsule within the glomeruli departed significantly from that of the fibrinoid prepared in the test tube and injected. The membranous, nodular, and capsular lesions were eosinophilic and hyaline and gave a positive (purple) reaction with the periodic acid-Schiff (PAS) procedure. Otherwise these lesions were nonsudanophilic and reacted like connective tissue elements with Mallory's aniline blue (blue), Masson's trichrome (green), and Mallory's phosphotungstic acid hematoxylin (orange-brown) stains. Moreover, these hyaline structures contained silver-positive fibrils.

The glomerular findings suggested that products from the autolyzed smooth muscle ("fibrinoid") induced the increased cellularity and the deposition of the membranous, nodular, and capsular substances.

The tubules contained casts with the characteristics of the injected fibrinoid and displayed focal degeneration, necrosis, or atrophy. The latter changes possibly resulted from vascular obstruction of the corresponding glomerulus.

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# Nonbacterial Diffuse Myocarditis Associated with Interstitial Pneumonia

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## Introduction

The etiological basis for diffuse interstitial or isolated myocarditis still remains unknown. However, an increasing number of reports have recently appeared in the literature mentioning certain complications, such as pulmonic inflammation, as a possible factor in causing this pathological process. Lyon,[1] in 1947, reported the occurrence of myocarditis in virus infection, mentioning that primary atypical pneumonia, which is an influenza-like virus disease, was occasionally accompanied by an acute myocarditis with electrocardiographic alterations. He also emphasized that from a clinical point of view importance should be attached to virus myocarditis connected with epidemic infectious hepatitis, infectious mononucleosis, yellow fever, varicella, mumps, etc.

Finland, Parker, Barnes, and Joliffe[2] have reported two cases of "influenza-pneumonia" with an acute myocarditis with necrosis of numerous muscle fibers accompanied by an interstitial infiltration of various types of cells. Stone[3] examined microscopic sections of the myocardium in 34 instances of lobar pneumonia and in 37 cases of bronchopneumonia and found polymorphonuclear leukocytic and round-cell infiltrations in the myocardium in 8.9% of the patients with lobar pneumonia. Among the patients with bronchopneumonia, acute focal myocarditis was found in 10.8%. Focal myocarditis associated with pneumonia was also mentioned by Neuhof,[4] Liebmann,[5] Berry,[6] Roesler and Soloff,[7] Swift and Smith,[8] and Saphir and Amromin.[9] However, a diffuse type of myocarditis was not mentioned in their series.

Fox and Overstreet[10] reported one case of myocarditis which was preceded by an upper respiratory infection, slight cough, and cyanosis. No bacteriological study was mentioned in their case report. In Greenebaum, Felson, and Zeligs'[11] case, cyanosis gradually developed and bronchopneumonia was found at autopsy.

Saphir[12] presented five cases of sudden death preceded by marked respiratory difficulty with findings of interstitial myocarditis. There was concomitant edema of the vocal cords and epiglottic region, which was regarded as evidence of early laryngotracheal bronchitis.

Covey,[13] in 1942, described a patient who died suddenly 14 days after a cold. The heart was enlarged and microscopically showed extensive and severe disease of the myocardium, the lesions being apparently of different ages. Covey[13] felt that the type of histological changes in the lungs and spinal cord favored a viral etiology. However, Covey[13] made no mention of bacteriologic or virus studies in his case report. Apparently no additional study has been carried out to substantiate his conclusion.

Myocarditis occurs frequently in acute poliomyelitis, having been observed in 14 of 35 cases of fatal poliomyelitis by Ludden and Edwards,[14] who considered that poliomyelitis virus is a possible cause of myocarditis. Myocarditis as observed in their series of cases was usually severer and proportionately commoner in adults than in young children.

Submitted for publication Oct. 16, 1957.

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Ungar [15] described "non-purulent myocarditis" in acute epidemic encephalitis, the myocardium containing infiltrations mainly of lymphocytes and of relatively few polymorphonuclear leukocytes. Lyon [1] stated that myocarditis accompanying virus disease may be of different degrees, ranging from severe fatal myocarditis during the acute stage of the virus infection to a sudden fatal outcome during the period of convalescence.

Helwig and Schmidt,[16] in 1945, isolated a virus from a group of anthropoid apes dying from interstitial myocarditis and were able to produce myocardial lesions consistently in mice.

Schmidt [17] subsequently reported the isolation of an agent from a chimpanzee dying of interstitial myocarditis, which produced myocarditis and encephalitis in mice and hamsters and myocarditis in guinea pigs. The myocardial findings in the heart were indentical with the myocardial lesions found in the human heart muscle in several virus diseases. Schmidt [17] also suggested that isolated myocarditis of man might be caused by virus.

Recently we have had the opportunity to study three cases of nonbacterial diffuse myocarditis associated with interstitial pneumonia. In view of the current study into the pathogenesis of nonbacterial diffuse myocarditis, it is believed appropriate to report the following three cases with review of the literature.

## Report of Cases

CASE 1.—A 2-year-old Negro boy was brought to the hospital with a short history and died four hours after admission. The information was obtained from the mother who stated that the baby was well until three hours prior to admission. The child had been coughing, with a slight fever, for approximately one month. No unusual drowsiness or irritability was noted prior to admission. The mother was a 35-year-old Negro who was sextigravida and sextipara; the pregnancies were normal. The baby was born at the hospital, and the delivery was normal. There was no serious trouble in the neonatal period.

Physical examination revealed a well-nourished Negro boy with marked respiratory difficulty and rapid pulse. The temperature was 38.5 C (101.3 F); the pulse, irregular, and the respiration rate, 40 per minute. The patient was cyanotic. His breathing was irregular and jerky. The heart beat was very irregular. The eyes were deviated to the right. There was no sign of icterus. Rales were heard over the entire right lung field. The extremities were flaccid, cold, and markedly cyanotic. There was no lymphadenopathy noted. The reflexes were hypotonic or absent.

The blood on admission showed a hemoglobin value of 13 gm. per 100 ml. and a leukocyte count of 20,500 per cubic millimeter, with 60% lymphocytes, 5% band forms, and 28% segmented polymorphonuclear leukocytes, 1% eosinophils, 3% metamyelocytes, and 2% myelocytes. The sickle-cell preparation was negative. The red cells were regular and well stained. Thrombocytes were adequate. The spinal fluid was not unusual. No urine was obtained. Roentgenological study showed extensive pneumonia, particularly in the base of the right lung. The child was running a temperature of 39 C (102.2 F); he received antibiotics and was subsequently placed in a high oxygen concentration but worsened steadily despite supportive therapy. He died four hours after admission. The clinical impression was acute cardiac failure due to endocardial fibroelastosis. Blood cultures were obtained at the ward.

Necropsy was performed three hours after death. The nasopharyngeal and oral cavities were not remarkable. No exudate was found. The heart weighed 80 gm. and was slightly dilated in both chambers. The endocardium was slightly pale, and the myocardium was extremely pale. No mural thrombus was present. The epicardium and pericardium were not remarkable. No valvular lesions were noted. The larynx and tracheobronchial passage was patent and free from any inflammatory edema or exudate. Both lungs were very heavy and were moderately hemorrhagic, with a firm consistency, particularly the right lower lobe, which was extremely hemorrhagic. The spleen was small and markedly congested. The remaining organs were also moderately congested. Postmortem cultures were made from the heart, lungs, spinal fluid, spleen, and lymph nodes, which were all negative, as well as the blood cultures taken on the ward.

Sections of the left and the right ventricle revealed diffuse interstitial infiltration of inflammatory cells composed primarily of lymphocytes, a few eosinophils and plasma cells, a small number of polymorphonuclear leukocytes, and a great deal of cellular debris. The muscular fibers were considerably necrotic (Fig. 1). Occasionally there were a few large mononuclear cells noted

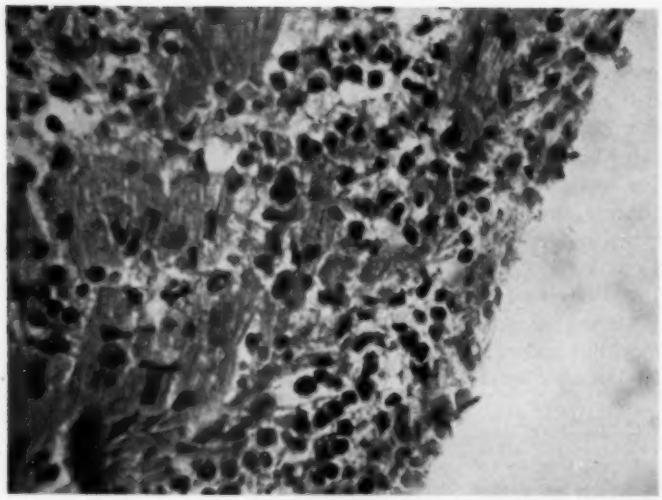

Fig. 1 (Case 1).—Section of left ventricle, showing necrosis of heart muscles and lymphocytic infiltration. Hematoxylin and eosin; reduced about 40% from mag. × 356.

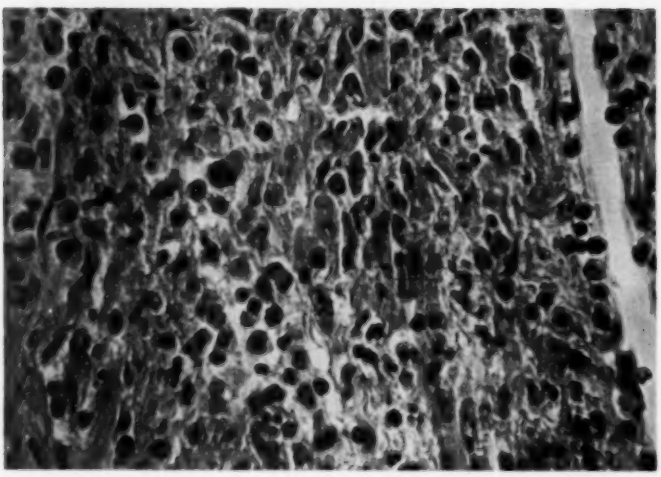

Fig. 2 (Case 1).—A few large mononuclear cells among the various types of inflammatory cells. Hematoxylin and eosin; reduced about 40% from mag. × 356.

among the inflammatory cells (Fig. 2). No Aschoff bodies were noted. The blood vessels showed no unusual changes. Sections of both auricles and of the septum revealed severe inflammatory changes, with marked necrosis of the muscles. The inflammatory cells were seen on the endocardium and also on the epicardium, diffusely infiltrated into the epicardial fat (Fig. 3). No lesions were noted in the valves.

Special stains for bacteria, fungi, and toxoplasma failed to reveal any organisms. The alveoli of the right lower lobe were practically filled with red cells. The left lung was moderately edematous. Sections of both lungs revealed marked thickening of the alveolar septi infiltrated with numerous lymphocytes, mononuclear cells, and a small number of polymorphonuclear cells.

Sections of the spleen and lymph nodes were not remarkable and showed only slight hyperplasia of the lymphoid tissue. Sections of the remaining organs revealed a moderate degree of congestion. Numerous sections of the brain and spinal cord showed no significant morphological alterations.

Case 2.—A 13-month-old Negro girl was brought to the City of Memphis Hospitals with fever,

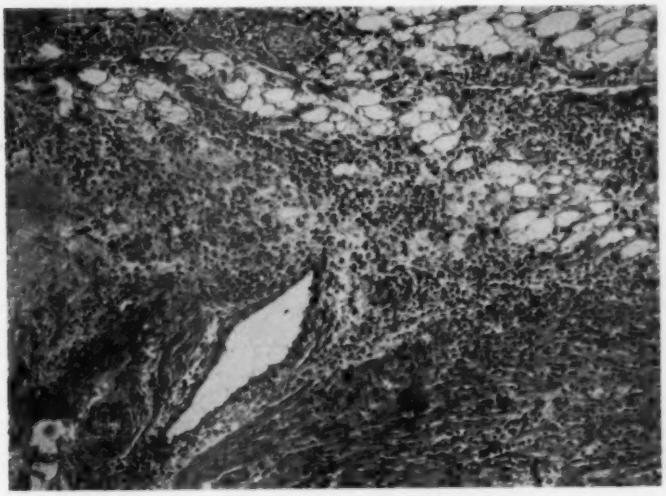

Fig. 3 (Case 1).—The epicardium is being diffusely infiltrated with inflammatory cells. Hematoxylin and eosin; × 160.

rapid respirations, cyanosis, and vomiting for approximately eight hours. According to the mother, the child had a slight fever for about two weeks, and the rapid respirations had begun approximately a week prior to admission.

Physical examination revealed a rectal temperature of 39 C, pulse of 152 per minute, and a respiration rate of 100 per minute. The heart beat was irregular on auscultation. Rales were heard over both lung fields. Roentgenological examination revealed severely consolidated lungs. The heart was enlarged. The liver was 5 cm. below the costal margin.

The laboratory data on admission showed a hemoglobin of 9.5 gm. per 100 ml. and a white cell count of 26,300, with 70% lymphocytes, 29% segmented neutrophils, and 1% band forms. The urinalysis was essentially normal. The child was given 0.12 mg. of digitoxin and 1,500,000 units of procaine penicillin and was subsequently placed in a high oxygen concentration. Response to treatment was transitory, and the pulse remained irregular. She died approximately two hours after admission.

Necropsy was performed two hours after death. The external examination was not unusual. Cultures were made from the heart, throat, and lungs. The tracheobronchial passage was patent and free from exudate; however, a scanty amount of mucus was noted in the bronchus. Approximately 50 ml. of clear translucent fluid was obtained from the pericardial sac. The heart weighed 85 gm. and was slightly dilated in the left ventricle. (Average weight of heart is 44 gm.) The epicardium and pericardium were not unusual. The myocardium was somewhat firm in consistency and gray. The endocardium was pale and slightly thickened. The valves were not remarkable. No mural thrombus was present. The right lung weighed 100 gm., being firm in consistency. A very scanty amount of fluid was noted on the cut surface. The left lung weighed 80 gm. and was also consolidated. No fibrous adhesions were noted. The spleen weighed 20 gm., and the white pulps were prominent. The mesenteric nodes were slightly enlarged but not otherwise unusual. The brain weighed 940 gm.

On microscopic examination the myocardium was diffusely infiltrated with lymphocytes and mononuclear cells, a small number of polymorphonuclear and plasma cells, a few eosinophils, cellular debris, and a few large mononuclear cells. The muscular fibers were disintegrated in many areas (Fig. 4). The inflammatory cells were also seen on the endocardium in both chambers. The epicardial fat was diffusely infiltrated with exudate (Fig. 5). No Aschoff bodies were present. The coronary arteries and veins within the heart were not unusual. The septum and both auricles showed similar changes to that in the ventricle. No valvular lesions were demonstrated. No pathogenic organisms were demonstrated. Sections of both lungs revealed the characteristic feature of an interstitial type of inflammation. The septi were considerably thickened, being infiltrated with many mononuclear cells and lymphocytes.

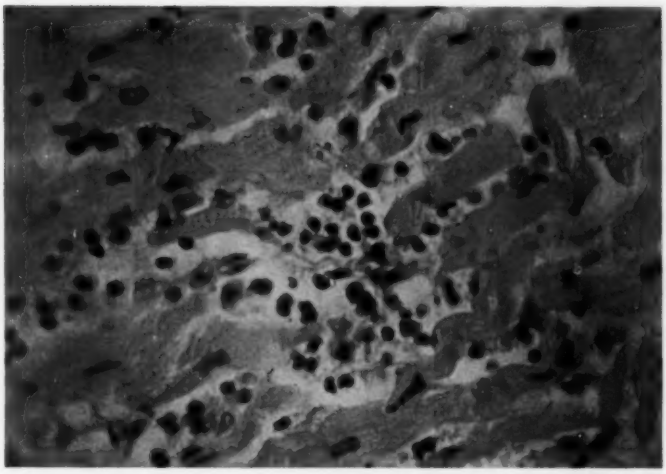

Fig. 4 (Case 2).—Many disintegrated muscular fibers are being infiltrated with inflammatory cells. Hematoxylin and eosin; reduced about 40% from mag. × 356.

A small number of polymorphonuclear cells were also noted, with a few giant-sized dark cells. The peribronchial regions were particularly thickened, being infiltrated with many mononuclear cells. Special stains failed to show any pathogenic agent. Sections of the spleen and lymph nodes revealed a moderate number of lymphoid cells without evidence of tissue necrosis. Sections of the liver, kidneys, and adrenals were moderately congested. Numerous sections of the brain and spinal cord revealed no significant morphological alterations.

Postmortem cultures of the heart, lungs, spleen, and lymph nodes failed to reveal any pathogenic organism.

Case 3.—A 2-year-old Negro girl was admitted to the isolation hospital with papular rash, high fever, and sickle-cell anemia. The child was well until three days prior to admission, when the mother noted a temperature elevation and swelling behind the ears. The sclerae became yellow-green. The child had been admitted on one earlier occasion for sickle-cell anemia and for pneumonia. There was no history of known contact with communicable disease.

Physical examination revealed an asthenic Negro infant with a temperature of 40.5 C (104.9 F) and

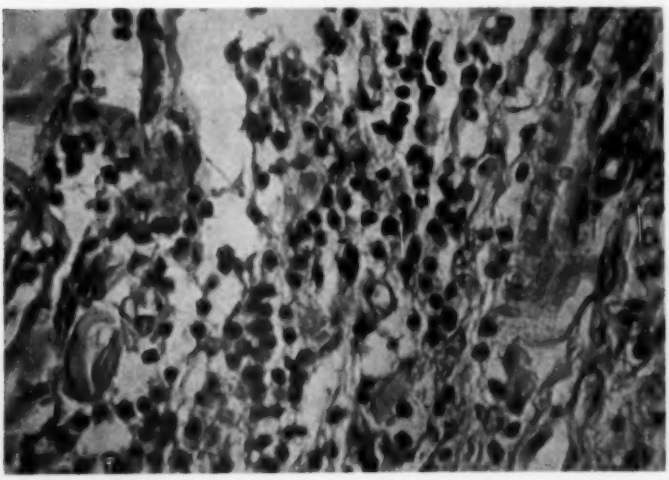

Fig. 5 (Case 2).—Epicardial fat, showing diffuse lymphocytic infiltration. Hematoxylin and eosin; reduced about 40% from mag. × 356.

pulse rate of 160 per minute; the mucous membranes were icteric. There was some pedal edema noted. The heart beat was irregular, and loud murmurs were heard over the apex of the heart. The lung fields were clear on percussion. The abdomen was flat and not remarkable except for the liver, which was felt 1 fingerbreadth below the costal margin.

The laboratory work revealed a hemoglobin of 5.0 gm. per 100 ml. and a white cell count of 40,900, with a differential count of 58% lymphocytes, 18% segmented forms, 24% band forms, and 5% nucleated red cells. The red blood cells were extremely hypochromatic, being sickled, with marked poikilocytosis. Many target cells were observed. The urinalysis revealed 2+ protein and a few white cells. The spinal fluid was not unusual. The temperature remained at 39 C until the day of death. A total of 500 ml. of whole blood and chlortetracycline (Aureomycin) were given during the hospital stay. However, the child died two days after admission.

Necropsy was performed six hours after death. The skin was moderately icteric. The oral and nasopharyngeal cavities were not unusual. The trachea and bronchi were not remarkable and were free from inflammatory exudate. The heart weighed 115 gm. and was slightly dilated in both chambers. (Average weight of heart is 56 gm.) The epicardium and endocardium were not remarkable. The myocardium was, however, very pale. No mural thrombus was noted. The right lung weighed 150 gm. and was firm in consistency, while the left lung appeared grossly hemorrhagic. The spleen weighed 26 gm. and was not unusual. The liver weighed 510 gm. and was light brown. The kidneys were of the usual size and shape. The remaining organs were moderately congested. Cultures of the blood and spinal fluid were negative. Additional cultures were made at necropsy on the heart, lungs, and lymph nodes, which were negative for pathogenic organisms.

Numerous sections of the heart revealed diffuse inflammatory changes in the myocardium, involving the most part of the left and right ventricles. The ventricular septum was also involved but was not severely altered in the muscular arrangement. Both atria and the atrial septum were also involved focally by the same processes. There were many lymphocytes and a few plasma cells noted in the epicardial fat and fibrous elements. The myocardial fibers were diffusely infiltrated with inflammatory cells composed of lymphocytes, plasma cells, a small number of polymorphonuclear cells, and a few large mononuclear cells (Fig. 6). Occasionally the muscle fibers were necrotic and disintegrated. The coronaries and the veins were not involved by the process. Beneath the endocardial lining the inflammatory exudate was associated with much cellular debris (Fig. 7). No bacteria or Toxoplasma bodies were demonstrated.

Sections of the lungs revealed a considerable degree of thickness of the septa infiltrated with numerous mononuclear cells and a few polymorphonuclear cells. The peribronchial areas were also infiltrated with

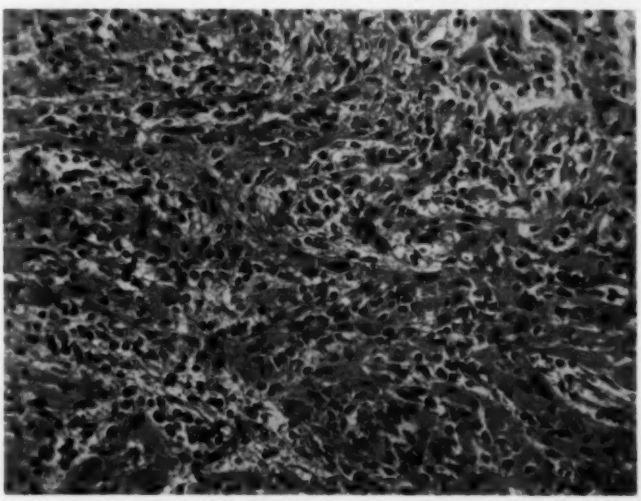

Fig. 6 (Case 3).—Section of the septum, showing a few large mononuclear cells. Hematoxylin and eosin; × 160.

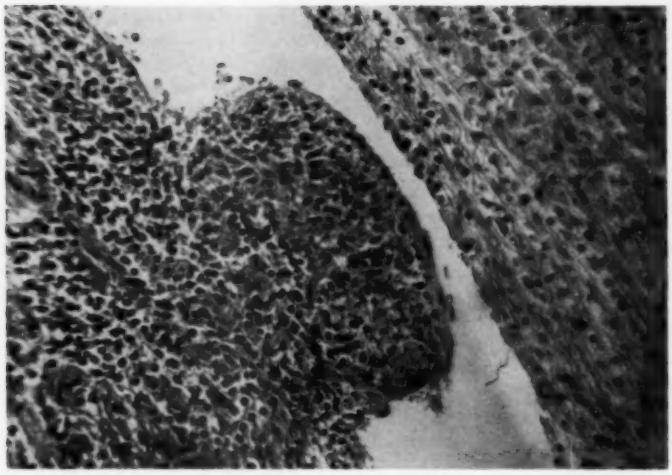

Fig. 7 (Case 3).—Section of the endocardium, showing diffuse infiltration of various types of inflammatory cells. Hematoxylin and eosin; reduced about 40% from mag. × 200.

numerous round cells and a few polymorphonuclear cells. There was marked hemorrhage noted in both lungs. Many macrophages were seen free in the alveoli. The bronchi were free of exudate, and the bronchial epithelial cells were relatively intact. The vessels were slightly engorged.

Sections of the liver revealed focal necroses, with many polymorphonuclear cells and round cells in the sinusoids. The cells were markedly swollen.

Sections of the spleen showed a moderate degree of congestion with slight hyperplasia of the lymphoid tissue.

Multiple sections of the brain and spinal cord failed to show any inflammatory changes.

### Cultural Methods

Cultures for the heart blood were done according to routine procedure using the brain broth, blood plate, and E. M. B. plates for both aerobic and anaerobic cultures. Cultures for the lung and spleen were done based on swab methods with use of the same media. Cultures for the lymph nodes were done specifically by using a piece of node for both aerobic and anaerobic conditions. The media was the same as the rest of the cultures. All cultures were kept for six weeks. The cultures for Brucella organisms from lymph nodes were done, but they were unsuccessful. No antistreptolysin titers were mentioned by clinicians, and we had not performed this similar test.

### Comment

In all the cases described, the pathological changes are consistent with those of a nonbacterial interstitial type of myocarditis. The myocardium, epicardium, and endocardium were diffusely involved by an interstitial exudate, consisting primarily of lymphocytes but with an occasional polymorphonuclear leukocyte, plasma cells, eosinophils, and large mononuclear cells. No Aschoff bodies were noted in these three cases. A considerable degree of muscular necrosis was readily demonstrated, but no valvular lesions were demonstrated. The cardiac lesions are morphologically identical with those described as isolated or Fiedler's myocarditis of unknown etiology. The significant features of our three cases described are (1) the extensive involvement of the endocardium and epicardium so that the lesions should be classified as pancarditis, (2) that all were associated with interstitial pneumonia in which the morphologic evidence suggests a viral etiology, (3) that all were preceded by respiratory infection followed by marked cyanosis and sudden death, and (4) that all the cultures were negative for pathogenic organisms.

Cultures from the heart, lungs, and spinal fluid and microscopic study of the sections

of the heart and lungs including Levaditi's stain and the preparations for toxoplasma failed to show pathogenic organisms in the three cases. This, of course, does not rule out the possibility that the micro-organism might have been present and not demonstrated. However, the repeated cultures gave no further information.

The involvement of the endocardium and the epicardium has not been mentioned by other investigators to our knowledge, except by House,[18] who described four cases of diffuse interstitial myocarditis which were accompanied by bronchopneumonia. The endocardium and epicardium showed only minimal involvement in his cases. All blood cultures were negative, although pathogenic organisms were cultured from the lungs. Kenny and Sanes[19] presented one case of diffuse myocardial inflammation in which a lobular pneumonia in the upper lobe of the left lung coexisted.

Lyon[1] stated that myocarditis accompanying virus diseases may be of different degrees, ranging from severe fatal myocarditis during the acute stage of the virus infection to a sudden fatal outcome during the period of convalescence.

In our cases described, the lesions of the heart which involved the entire layers of the heart seemed to be preceded by the interstitial pneumonia, which on morphological and bacteriological evidences is presumably caused by a virus infection. However, the true nature of the viral myocarditis will have to be the subject of future study.

## Summary and Comment

Three cases of nonbacterial pancarditis which are morphologically identical with those of isolated or diffuse interstitial myocarditis of unknown etiology are presented which occurred in three children under 2 years of age. The patients had previously developed fever, rapid respirations, and cough prior to hospital admission, followed by marked cyanosis and sudden death. A moderate degree of interstitial pneumonia was noted in each case, which on morphological evidence was probably due to a virus infection. No pathogenic organisms were demonstrated by the repeated cultures of the blood, lungs, spinal fluid, and spleen and by microscopic studies of the sections of the heart and lungs.

The data in these cases would suggest that a virus infection may be linked with myocarditis which involved the entire heart. The occurrence of an acute, subacute, and nonbacterial diffuse myocarditis in pneumonia is briefly reviewed from the literature.

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# The Role of the Vertebral Venous Plexus in the Dissemination of Labeled Emboli

JOSEPH P. WACK, Ph.D.; THEODORE DUBUQUE, M.D., and JOHN P. WYATT, M.D., St. Louis

The demonstration of the vertebral plexus by Batson[1,2] by injection of radiopaque material into cadavers awakened interest in the potential role this primitive pathway possibly played in the dissemination of embolic material. The observation often recorded in autopsy protocols that primary malignant growths adjacent to the caudal portion of the vertebral axis often produced remote metastases in organs such as the lung or brain was readily accepted as support for Batson's anatomical assumptions. The influence of the vertebral venous system on the spread of Walker rat carcinoma was also clearly implied in Coman's experimental studies.[3]

To determine more accurately the role of the vertebral venous plexus in the dissemination of foreign material and ultimate sites of lodgment of this embolic material, in vivo tracing with radioisotopic-labeled emboli has been introduced in an attempt to give a pathogenetic appraisal of this venous route that was not possible previously with conventional morphologic methods.

## Materials and Methods

A donor dog, a normal adult male mongrel, was given 4 ml. of ferrous ascorbate intravenously. Each milliliter contained 0.039 mg. of elemental iron having an activity of 100$\mu$. These injections were given monthly during the course of the experiments in order to maintain the radioactive-iron content of the red cells at a consistently high level. The uptake of iron and cycle of incorporation into the canine red blood cells followed the known metabolic patterns.

*Clot Preparation.*—As needed, 30 to 60 ml. of blood was withdrawn by cardiac puncture from the donor dog, poured into a flat dish, and allowed to clot and dry. When the dried blood became brittle it was ground in a mortar and pestle and passed through a wire mesh of known grid measurement. These wire meshes were of the conventional type, such as are used in estimating dust particle size in mineralogical surveys. Clots with an area of from 500$\mu$ to 1000$\mu$ were most frequently used. It was determined that sizes under 500$\mu$ were trapped inevitably in the small vessels in the caudal portions of the vertebral plexus, while larger clots of over 1000$\mu$ were not able to pass through the cervical portion of the plexus.

*Surgical Technique.*—The experimental dogs were prepared by ligating the inferior vena cava and the azygos veins. The animals were given intravenous pentobarbital anesthesia, with a mechanical respirator in use during the opening of the thorax. The caval ligation was done through a lower midline abdominal incision. The inferior vena cava was isolated below the renal veins, doubly ligated, and divided. Stripping of adventitial tissue posterior to the vena cava was carried out to the inferior level of the diaphragm. The azygos vein was approached through the fifth or sixth interspace on the right, ligated, and divided near the superior vena cava.

After a lapse of from one to five days, the right femoral vein was exposed on the experimental dogs, with use of pentobarbital (Nembutal) anesthesia. One gram of the dried radioactive blood clots was suspended in isotonic saline to facilitate injection. With use of a large-bore plastic tube, the labeled emboli were immediately injected into the animals. The average radioactive count of the embolic dose was in the range of 60,000 cpm. The experimental dogs were killed after five minutes by injection of anesthetic ether into the heart. The radioactive "emboli" were injected while the animals were in various positions: the supine position, lying on their left sides, lying on their right sides, the erect position (head up), and suspended by their feet (head down).

Submitted for publication Nov. 6, 1957.

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This investigation was supported in part by Grant A-1597 from the Department of Health, Education and Welfare, U. S. Public Health Service.

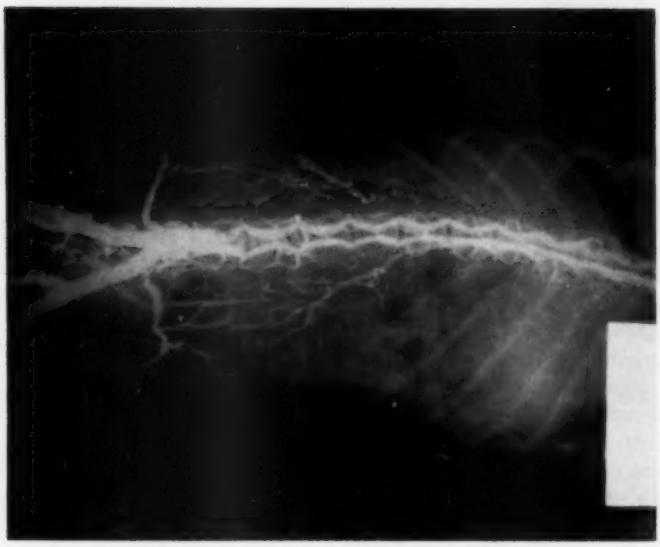

Fig. 1.—Anteroposterior angiogram of the experimental dog. Note site of ligation of inferior vena cava and distended paravertebral plexus, with many collaterals in lumbosacral region.

Angiograms on the experimental animals were obtained two to four days after operation via a right femoral venous cut-down, using 20 cc. of 70% acetrizoate (Urokon) in order to visualize the vertebral plexus and its collateral tributaries, (Figs. 1 and 2).

The lungs, the vertebral plexus, and the remaining viscera were independently scanned in order to obtain the distributional pattern of the injected emboli. A high efficiency γ-tube (Tracerlab TGC-8) was used.

The lungs were separated into their anatomical lobes, and the lobes were cut into sections of uniform size. The radioactivity in these sections was determined by means of a deep-well scintillation counter; the geometric calculations were kept constant throughout the experiment.

A number of the control animals (Figs. 3A and 3B) and experimental animals (Figs. 4A and 4B) were given injections of the radioactive clots; the entire lung was removed, fixed in formalin, infiltrated with gelatin, and frozen, and macro-

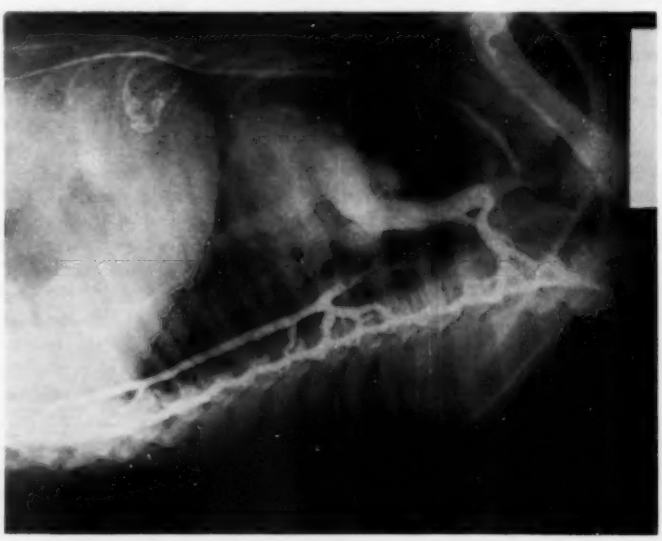

Fig. 2.—Lateral angiogram of experimental dog. Note site of ligation of the azygos vein and distended paravertebral plexus. Angiograms taken two to four days after vessels were tied off.

Figure 3

Fig. 3A.—Section of lung from unoperated-on dog after injection of radioactive "emboli." Lung embedded in gelatin and cut at 300$\mu$.

Fig. 3B.—Gross autoradiograph from lung section shown in Figure 3A. Note diffuse pattern of emboli. Lung exposed three weeks on medical x-ray film.

sections 300$\mu$ thick were cut. These sections were placed on medical x-ray film and exposed for three weeks, after which time they were developed in the normal manner.

## Results

It was noted that with the standardized blood "clots" below 500$\mu$ in size, these labeled clots remained confined to the lower third of the paravertebral venous plexus and spread out, as Batson had noted with his extremely thin injection mass, into the veins in and about the sacrum and the lower cutaneous abdominal veins of the flank and ventral surface. With emboli above 1000$\mu$, they remained confined to the paravertebral region, although scanning revealed counts of significance in the lower cervical region. Scanning of the skull bones and brain failed to reveal in any of the test animals radioactive counts of significance.

Prior to injection of the graded emboli, the total radioactive count was determined. In this manner, the systemic partition of the labeled emboli was readily determined. With emboli varying in size between 500$\mu$ and 1000$\mu$, it was recorded that, on the average, one-third of the radioactive emboli were contained in the thoracolumbar portion of the vertebral venous system; one-third, within the lungs, and the remaining one-third of the embolic material was divided between the liver, the mesentery root, and the kidneys. It is our contention that the minute radioactive emboli reached these latter three anatomical regions through venous communications rather than by transpulmonary passage. Sampling of cardiac blood at the time of death failed to reveal radioactivity.

In the control animals, all radioactivity was confined to the lungs. Blood and organs

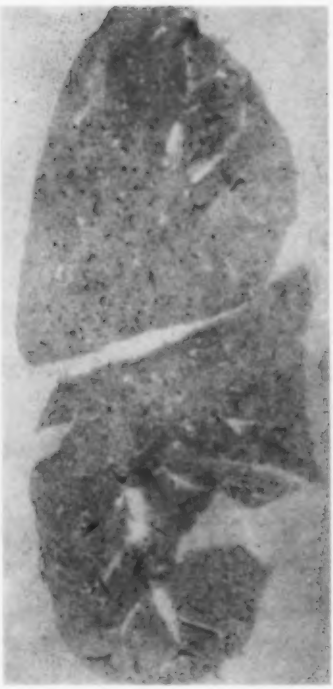

Figure 4

Fig. 4A.—Section of lung from experimental dog, embedded in gelatin and cut at 300μ.

Fig. 4B.—Gross autoradiograph from lung section in Figure 4A. Note embolic areas in the apical region, indicated by increased reduction of x-ray film.

sampled throughout the body were free of radioactive embolic material.

With conventional methods, any loss of emboli or visual uncertainty introduces a sizable error into any study of embolism which concerns either routes of spread or sites of particle lodgment. Extended studies now under way on experimental pulmonary embolism, in which the tagged emboli are introduced into femoral and jugular veins, have confirmed the superiority of this technique over orthodox morphologic methods.

The difference in the clinical behavior of the two groups of animals was startling. In the control group, with direct introduction of the emboli into the femoral-caval pathway, the animals died suddenly of acute pulmonary embolism, with a diffuse distribution of emboli in both lungs. The syndrome of acute pulmonary embolism was not observed in the experimental animals.

The anatomical distribution of emboli to the lungs and within the individual lobes is presented diagrammatically in Figures 5 and 6. The favored distribution of the right lung over the left was noted in both controls and experimental groups in the plantographic position. The only reversal of this response was observed in animals stretched out in the supine position. With the animals fastened on the right side, the bases of both lungs, particularly the posterior portions, had the greatest concentration of emboli. With the animals on their left side, the tendency was for the emboli to be swept to the apical portions of the lung. With the animals suspended in a perpendicular fashion, apices of the right lung were favored and the bases of the left lung.

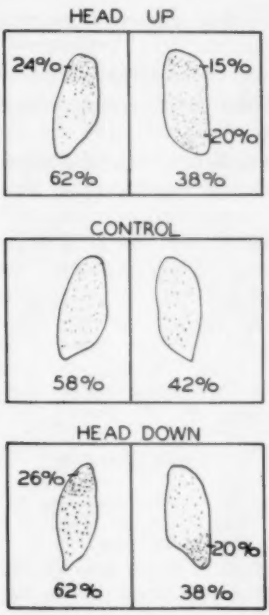


Fig. 5.—Positional influence and distribution of labeled "emboli."

This finding was unrelated to the cephalic position of the animal.

## Comment

A cautious extrapolation of these findings to human pathology is necessary, but several basic facts emerged which offer a framework of discussion on the problem of embolism. The right lung is larger than the left in mammals, and the tendency for more emboli to lodge in the right lung has long rested on this anatomical canon. The accentuation of this finding in experimental dogs suspended by their forelegs or hindlegs emphasizes the primary importance of the volume of blood flow. However, the possibility that this contrapolar distribution in which the greater concentration of embolic material was noted in the apices of the right lung and bases of the left lung, with the experimental dogs suspended respectively from forelegs and hindlegs, might be related to anomalous azygos vein distribution, rather than to gravitational influences, cannot be eliminated.

The lodgment of these minute emboli in the lung, by these in vivo studies, clearly demonstrated that the lungs are connected with the paravertebral plexus and offered one explanation for the appearance of remote metastases in malignant tumors arising and invading the pelvic paravertebral region. The size of emboli as well as survival rate determine to a certain extent the localization or disseminative pattern of the disease, and this may be particularly true of the study of malignant tumors.

From the control group it was clearly demonstrated that with the injection of minute radioactive emboli into the caval system all emboli reached and were retained within the pulmonary circulation, although transpulmonary passage has been held to be possible via pulmonary arteriovenous shunts. The distribution of emboli was equally diffuse throughout the two lungs. As far as localization of emboli was concerned there was no significant difference between peripheral and central portions of the lungs. The peripheral distribution of these small emboli being well out to the costal margins of the lungs, as depicted in the gross autoradiogram, supported the contention that the intrinsic pattern of the pulmonary vasculature may be of the main-line type and influence the distributional patterns of minute emboli, even when the route of entry is other than directly through the jugular-caval channels. These findings are strikingly similar to those noted in postmortem studies on persons dying from showers of minute emboli.

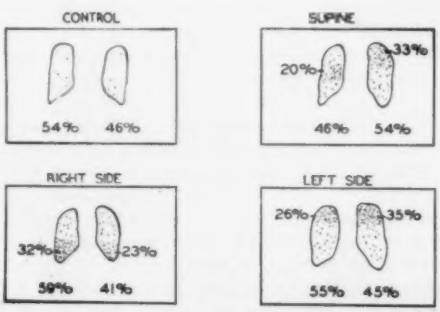


Fig. 6.—Positional influence and distribution of labeled "emboli."

In contrast to diffuse embolism with minute emboli, in man the common form of embolism is associated with large emboli, which are influenced by gravitational effects and volume of flow. The large emboli lodge in hilar main-line branches and often occlude branches in the right lower segments of the lung. This form of embolism has not been undertaken as yet by the methods described in this contribution.

As a final tenet, the use of dehydrated blood clots as embolic material eliminated to a large extent the criticism which has been directed against heavy-density emboli, such as lead shot,[4] as used by Steinberg and Mundy[5] or glass beads of Prinzmetal type.[6] The specific gravity of these radioactive clots approached more closely the specific gravity of blood than is possible with metallic emboli. The gravitational influence noted in other studies with heavier emboli invalidates to a large extent their use in experimental studies of embolism.

## Conclusions

Labeled emboli made from dehydrated canine blood in which radioiron had previously been incorporated in vivo, measurable as to size and radioactivity, offer an extremely useful means of following pathways of dissemination and tracing terminal sites of lodgment in experimental investigations concerning embolism. Irrespective of the route of injection, whether along caval or vertebral venous channels, these studies revealed that the right lung because of its size received a greater proportion of the emboli than the left. Perpendicular suspension of the experimental animals enhanced this lung partition. Lobar distribution within the lungs was influenced by the lateral positioning of the test and control animals.

The filtering capabilities of the vertebral venous system, with particular reference to size of emboli, were noted. The prominence of the lung as the end-organ for emboli introduced into the vertebral system was a noteworthy feature of this experimental study.

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# Adenoacanthomas of the Colon

C. BARRIE COOK, M.D., and G. D. KLICKSTEIN, M.D., Washington, D. C.

Adenoacanthomas of the large bowel are felt by some to be fairly common. Especially is this true in regard to the rectum. In reviewing the material of the Mallory Institute of Pathology of the Boston City Hospital for the past 11 years, we have found 12 records of patients with adenoacanthoma in the autopsy file and 53 records in the surgical file. During this same period of time there were 10,014 autopsies and 61,000 surgical cases. This represents an incidence of about 0.1% in the autopsies and about the same percentage in the surgical cases. The locations of these may be seen in Tables 1 and 2.

We would like to comment in detail on those in our series occurring in the large bowel, as the number of these in the literature is few. We feel that adenoacanthomas of the colon are probably commoner than the literature leads one to believe. A total of seven adenoacanthomas in the present series were located in the gastrointestinal tract; five of these involved the colon.

For this reason we feel that these cases merit reporting.

CASE 1.—A 70-year-old white man was admitted with chief complaint of diarrhea and constipation of three months' duration prior to admission. During this time he noted spotting of blood per rectum and weight loss. Past history was of interest only in the report of a negative rectal examination three years prior to admission.

Physical examination revealed a nodular right upper quadrant mass which moved with respiration and was thought to be liver. Rectal examination revealed a mass 3 in. from the anus, which was soft, irregular, and polypoid. Biopsy of this mass revealed a malignant tumor growing in part as a adenocarcinoma and in part as a squamous carcinoma. A transverse colostomy was performed, at which time he was found to have liver and peritoneal metastases. Biopsy of one of these revealed mucin-producing adenocarcinoma.

CASE 2.—A 69-year-old white man was admitted with the chief complaint of diarrhea six to eight times per day for the past six months. The stools were loose and at times contained bright red blood. He was seen by his family doctor three months prior to admission, who found "something" on rectal examination, but the patient did not return for follow-up examination. During this six-month period the patient noted a 10 lb. weight loss. During the week prior to admission he experienced a severe episode of lower abdominal pain.

Physical examination revealed the following positive finding: a large polypoid mass on rectal examination fixed to anterior wall at the tip of the examiner's finger.

Biopsy of this mass revealed an adenoacanthoma. A Miles resection including perianal skin was performed. Four centimeters proximal to the anorectal junction was an annular fungating ulcerated lesion nearly encircling the bowel wall. On section it was gray, granular, and fairly firm and extended through the bowel wall.

Submitted for publication Nov. 4, 1957.

From the Mallory Institute of Pathology, Boston City Hospital, Boston 18.

TABLE 1.—*Autopsy Cases of Adenocanthoma*

| Location | Cases, No. |
|---|---|
| Lung | 3 |
| Gallbladder | 1 |
| Uterus | 2 |
| Pancreas | 2 |
| Stomach | 1 |
| Larynx | 1 |
| Prostate | 1 |
| Bladder | 1 |
| Total | 12 |

TABLE 2.—*Surgical Cases of Adenoacanthoma*

| Location | Cases, No. |
|---|---|
| Uterus | 31 |
| Ovaries | 4 |
| Cervix uteri | 3 |
| Vagina | 1 |
| Lung | 2 |
| Stomach | 1 |
| Esophagus | 1 |
| Bladder | 2 |
| Gallbladder | 1 |
| Neck | 1 |
| Lymph node | 1 |
| Rectum | 4 |
| Transverse colon | 1 |
| Total | 53 |

A little over one year later on readmission a perineal tumor mass was found which on biopsy proved to be an adenocarcinoma.

His last admission in a terminal state was two years four months after his first admission. No autopsy was obtained.

CASE 3.—A 69-year-old white man entered with the chief complaint of aching lower abdominal pain for the past eight months, intermittent in character and not affected by changes in position or bowel movements. During the past two months it became severer and he developed loose bowel movements, up to five or six per day. Some of these contained fresh blood and small clots. He noted a weight loss of 55 lb. in the previous six months, as well as easy fatigability.

Physical examination revealed significant positive finding on rectal examination of a hard mass 2½ in. from the anus on the posterior wall. Gross blood was present on the examiner's finger.

Biopsy of the mass was done and revealed squamous metaplasia of glands and one area of adenocarcinoma invading the stalk.

After this the patient signed out against advice, to return approximately two months later with a history of similar but more marked complaints than on his first admission. The mass was again felt on rectal examination and seemed to be larger. Two days after admission a transverse colostomy was performed for obstruction. Postoperative diagnoses of peritoneal and hepatic metastases were made. Sigmoidoscopy several days later showed lesions at 2 and at 9 in. Findings on biopsy of these were reported as invasive mucin-secreting adenoacanthoma. Several days later the patient died, and no autopsy was obtained.

CASE 4.—A 54-year-old white man was admitted with the diagnosis of a right-lower-quadrant pain of three days' duration which was worse on moving.

The only positive finding on physical examination was a mass in the right lower quadrant which was firm and not movable. Rectal examination was negative.

Two days after admission a transverse and right colectomy was performed, with anastomosis between the terminal ileum and descending colon. At operation the patient was found to have a tumor of the transverse colon, with perforation and abscess formation and with the abscess contained in the gastrocolic omentum. No metastases were found. The diagnosis on this specimen was adenoacanthoma.

Following the operation he developed bilateral subdiaphragmatic abscesses which resulted in a very stormy postoperative course and in the patient's death on the 16th postoperative day.

An autopsy was performed and revealed no evidence of residual tumor.

CASE 5.—A 46-year-old white man was admitted with the chief complaint of an episode of involuntary defecation with the passage of a cupful of blood and three to four pieces of tissue "resembling kidney." These were about the size of his thumb. This occurred about five to six weeks prior to admission and was not associated with any other symptoms. He went to the outpatient department and was given an appointment for the following day but did not show up. In the three to four weeks prior to admission he noticed that his stools became ribbon-like.

The important findings on physical examination were a 2 by 8 cm. cylindrical mass in the left lower quadrant which was nontender and the presence of a mass felt on rectal examination on the posterior wall at the tip of the examiner's finger.

Sigmoidoscopic examination revealed a mass at 10 cm. Biopsy of this mass revealed adenocarcinoma. A Miles resection was carried out, and the tumor was found 13 cm. from the anus; on section the tumor was fibrous and gritty, with numerous areas of soft yellow necrotic-appearing material. Microscopically this was found to be an adenoacanthoma.

A brief review of the published cases is presented to show the rarity of this lesion in the colon. Special emphasis is given to the number and type of metastases where noted. Probst [12] reported an adenoacanthoma of the sigmoid colon in a 60-year-old man in which all of the metastases were adenocarcinoma.

Herxheimer [4] described a surgical case in which no history was given. The tumor originated in the cecum and was largely a colloid carcinoma with squamous elements. There were no metastases reported.

Plenge [11] reported an adenoacanthoma of the ileocecal valve in a 28-year-old woman. This one showed metastases to the nodes in the immediate vicinity and one in the pouch of Douglas. All metastases showed both squamous and glandular elements.

White and Brunton [17] reported briefly on a 64-year-old man with a large malignant ulcer in the upper rectum infiltrating the small bowel and pelvic tissue. Microscopically, it proved to be largely an adenocarcinoma associated with active tuberculous granulomata and a few small nests of squamous carcinoma. Two small metastases were found in the liver, one purely adeno-

carcinoma and the other purely squamous carcinoma.

Rabson [13] reported an adenoacanthoma in the ascending colon just distal to the cecum in a 49-year-old woman. The tumor involved a large segment of the colon and penetrated through the wall to involve the small bowel; there were no metastases present.

One of the Cabot cases [6] in *The New England Journal of Medicine* reported on a 70-year-old woman whose pelvis was frozen by tumor which proved to be an adenoacanthoma. The mucosa over the involved area of sigmoid colon was intact, making it doubtful that the tumor arose from the bowel. In the discussion, Dr. Tracy Mallory referred to an example from his own experience that had arisen in the colon.

Schmidtmann [16] described a squamous carcinoma of the ileocecal valve in a 65-year-old woman. Though this tumor does not properly belong in the category of adenoacanthomas, it is interesting from the standpoint of etiology. He ascribed the etiology to metaplasia.

Goncalves [3] reported on a number of adenoacanthomas arising in various locations, but only one of his was located in the bowel, and it was located 5 cm. from the anus.

## Comment

Tumors comprised of glandular and squamous epithelium are uncommon tumors that are referred to in the literature by various names: polymorphous epithelioma, adenocancroid, Malpighian epithelioma, and adenoacanthoma. Considerable interest attaches to the genesis of the heterologous pattern of growth in these neoplasms. Herxheimer,[4] who was first to describe such a tumor, in 1907, considered both the squamous and glandular elements to be malignant. On the other hand, others consider these neoplasms as adenocarcinoma with foci of nonmalignant squamous metaplasia.

According to the literature, the commonest location of these tumors is in the female genital tract. Our findings verify this fact. According to Novak,[10] the site of greatest incidence in the female genital tract is in the body and fundus of the uterus, where they arise in the endometrium. Adenoacanthomas have been reported as arising in many different areas, e. g., cardioesophageal junction,[15] stomach,[19] ovaries,[2] pancreas,[7] thyroid,[14] uterus,[8] and gallbladder.[5] A few from other areas are also reported, but much less commonly.

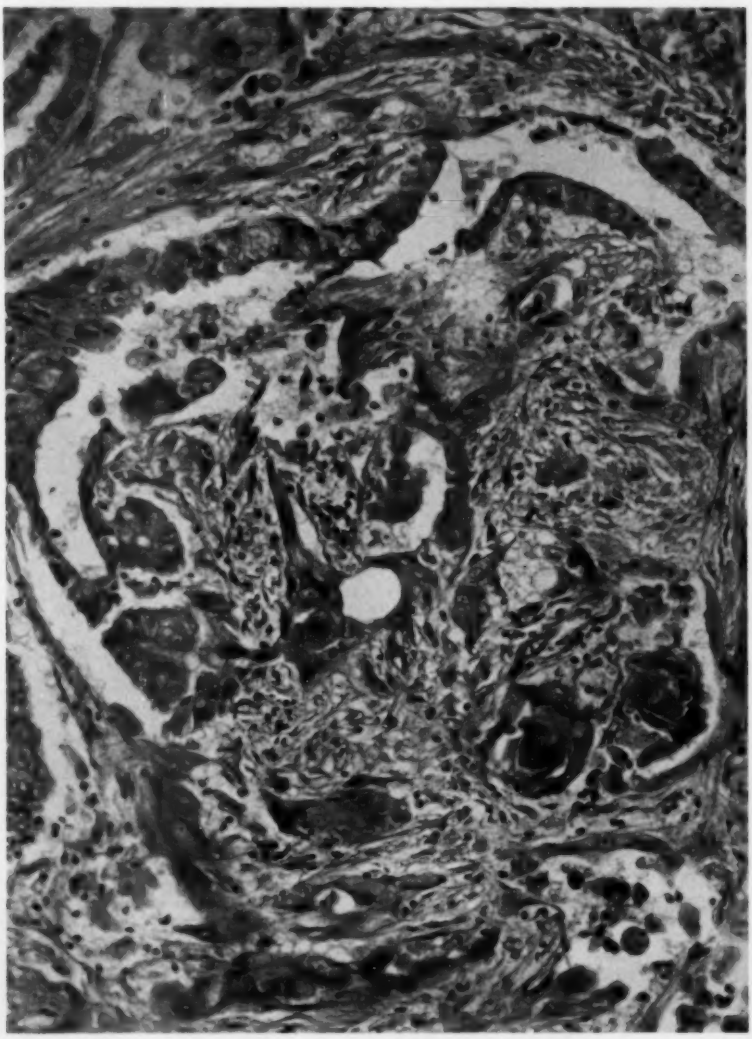
From reviewing those reported in the literature as well as our own cases, we find that there are no gross characteristics enabling one to suspect or make a gross diagnosis of adenoacanthoma (Fig. 1). It is only when microscopically the tumors are found to be growing in part as squamous carcinoma and adenocarcinoma that a positive diagnosis of adenoacanthoma can be made (Figs. 2 and 3). We feel that it is essential for the diagnosis that both portions of the tumor be malignant. An adenocarcinoma may have small foci of nonmalignant squamous metaplasia associated with it. This does not make this type of tumor an adenoacanthoma.

Fig. 1.—Adenoacanthoma of transverse colon, causing constriction due to annular growth.

However, it may be on its way to becoming an adenoacanthoma. More sections of an adenoacanthoma with focal areas of nonmalignant squamous metaplasia may reveal other areas where it has become malignant. Review of the metastatic patterns of the series of tumors here reported supports the view that both the glandular and the squamous elements are malignant.

In the 12 examples of this type of tumor that came to autopsy in our series, 3 failed to show metastases but did extend locally. Both the squamous and adenocarcinoma portions of the tumor were present in this local spread. Of these three, one was located in the gallbladder; one, in the bladder, and one, in the prostate. Of the other nine that did show metastases, four had only mixed

Fig. 2.—Section of adenoacanthoma of transverse colon, revealing tumor to be growing in part as squamous and in part as adenocarcinoma. Hematoxylin and eosin; reduced 13% from mag. × 400.



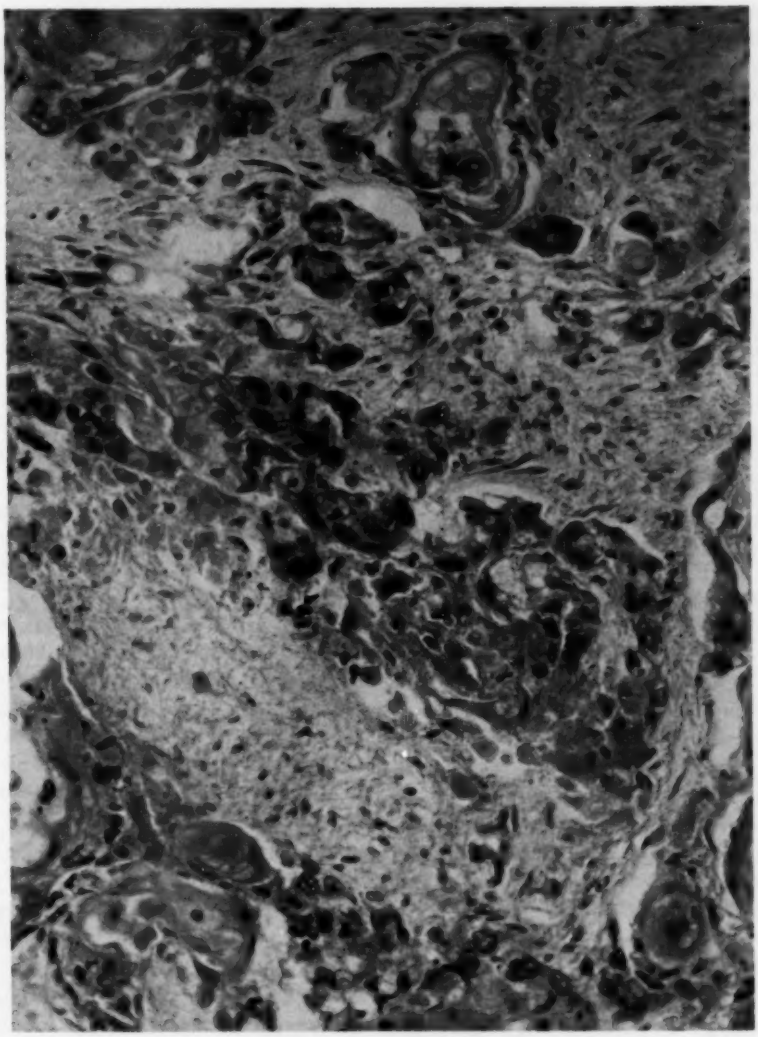


Fig. 3.—Another section of adenoacanthoma of large bowel, revealing this portion to be largely undifferentiated or squamous carcinoma. Hematoxylin and eosin; reduced 13% from mag. × 400.

adenocarcinoma and squamous metastases, two had only squamous metastases, two others had pure squamous and mixed (adenocarcinoma and squamous) carcinoma, while the last one had pure adenocarcinoma as well as areas of mixed (adenocarcinoma and squamous) carcinoma. That this variation in the type of metastatic lesion occurs was previously noted in reviewing those reported arising in the large bowel. Each of ours that came to autopsy had only one primary tumor as a source for the metastasis, thus refuting the argument of those who might feel that the variation in types of metastases was due to two separate lesions. It is possible in the surgical series that two separate primary lesions may have been present, but since this was not found to be true in our autopsy series this possibility seems unlikely.

As one looks over the origin of adenoacanthomas in our series, it may be seen that they tend to arise mainly under one of two circumstances: first, where squamous and glandular epithelium are contiguous, and second, in those organs where there is frequent squamous metaplasia. This had led many to ascribe different modes of origin to these tumors, depending on their site.

The origin of heterologous growths has always been of great interest. Of the many theories, four have received the most attention. They are (1) embryonic rests, (2) overgrowth of neighboring epithelium, (3) squamous metaplasia, and (4) prosoplasia (a pathologic change in the epithelium manifested by further differentiation from the surrounding epithelium). Excellent discussions of these theories may be found in articles by Herxheimer,[4] Plenge,[11] Nochimowski,[9] and, more recently, by Wood.[19] Wood [19] previously noted the location of adenoacanthomas and presented experimental data to corroborate his thesis that these tumors arise by direct stimulation of "undifferentiated basal cells."

We feel that, of the above theories, squamous metaplasia explains the origin of adenoacanthomas in any location. In going over the slides in our series, some lesions were found to be composed of similar amounts of squamous carcinoma and adenocarcinoma. Slides of other cases revealed the primary tumor to be composed almost entirely of poorly differentiated adenocarcinoma except for small foci of squamous metaplasia. Metastases showed more squamous elements than the primary, which suggests that in some instances the metaplasia might occur in the metastatic lesions. This could be explained on a pressure basis as with the cystic ovarian adenoacanthomas.

As previously mentioned, 9 out of 12 cases which came to autopsy showed metastases. Seven of these nine cases had metastases composed of both elements. This indicated to us that both portions of the tumor are malignant (Figs. 2 and 3).

In an analysis of the malignancy of these tumors in various locations, Calderara [1] and Nochimowski [9] have stated that they vary little from adenocarcinomas of the same location. Our findings on the malignancy of adenoacanthomas are in agreement with theirs. In speaking of adenoacanthomas of the uterus, Willis states that they behaved as other endometrial adenocarcinomas.

## Summary

Sixty-five cases of adenoacanthoma from the surgical and autopsy files of the Mallory Institute of Pathology of the Boston City Hospital are presented. The incidence in this series is approximately 0.1%, with the greatest number occurring in the uterus. Special emphasis is given to five occurring in the large bowel, with a brief review of the literature on large-bowel adenoacanthomas.

Adenoacanthomas are tumors composed of squamous and glandular elements, both of which are malignant and may metastasize. Nine of the twelve autopsied cases of adenoacanthoma showed metastases of one or both elements. There are no special features of these tumors which enable one to make a gross diagnosis.

The sites of occurrence of these tumors are noted, and the theories as to the etiology are discussed. We favor that theory which designates the origin deriving from squamous metaplasia. Their behavior pattern is felt to be similar to that of well-differentiated adenocarcinomas of the same location.

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## Addendum

Since the completion of this paper, three more adenoacanthomas have been seen by us. One was in the uterus; one, in the pancreas, and the third, in the rectum. The last case came to autopsy and had metastatic lesions composed of both elements.

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## CORRECTIONS

In the article "The Interrelationship Between Cortisone and Parathyroid Extract in Rats," by Drs. Zvi Laron, Jean P. Muhlethaler, and Robert Klein, in the February issue of THE ARCHIVES (p. 125) the photomicrographs comprising the upper part of the Figure were printed in reverse order. The section labeled *A* should be *D*, *B* should be *C*, *C* should be *B*, and *D* should be *A*. The legends are correct as printed.

In the article "Cells of Human Heart and Aorta Grown in Tissue Culture," by Estelle Fasolino Ingenito, Ph.D., and co-authors (April issue, p. 355), the legend for Figure 1 (p. 357) should read: "Some typical heart tissue cells (eighth transfer, routine technique). Hematoxylin and eosin."

# Studies on the Nature of the Abnormal Fibers in Pseudoxanthoma Elasticum

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Although the clinical and histologic changes in the involved skin in pseudoxanthoma elasticum were clearly described decades ago, the etiology and pathogenesis of the disease are still obscure. One issue that has received considerable attention in the literature is whether the involved fibers in the skin are derived from collagen or from elastic tissue. The purpose of this report is to present the results of elastase digestion, microincineration, and electron microscopy, as well as standard histologic methods, in the study of skin from a patient with pseudoxanthoma elasticum. These results, although they do not clarify the etiology of the condition, suggest a pathogenic concept of the disease which may explain the varied opinions regarding the nature of the involved fibers.

## Present Study

### *Materials and Methods*

The material studied consisted of two pieces of skin from the axilla of a 49-year-old woman with classical pseudoxanthoma elasticum. One biopsy specimen measuring 1.4×0.5×0.3 cm. was fixed in 4% formaldehyde and embedded in paraffin. Sections were stained with hematoxylin and eosin, Lillie's modification of the Masson trichrome stain, and Verhoeff and orcein elastic tissue stains. Certain sections were exposed to elastase (Merck) digestion at pH 8.4 for periods of 45 minutes and 2 hours at room temperature, after which they were stained with orcein in the usual manner. Normal human elastic tissue obtained by biopsy of skin and fixed in formalin and purified elastin from beef ligamentum nuchae[1] were also digested with elastase as above for control purposes.

Sections cut from this formalin-fixed and paraffin-embedded material were also microincinerated and dry-mounted for dark-field visualization.

The second piece of skin was fixed in buffered osmic acid and embedded in methacrylate[2] for electron microscopy. Some relatively thick sections were cut from this block for phase-microscope examination in order to enhance orientation of structural elements. The Philips EM100B electron microscope was used in these studies at varying magnifications, and the experimental material was compared with normal elastic tissue and normal collagen from human skin.

### *Results*

Histologic examination of hematoxylin-and-eosin-stained slides revealed the characteristic changes of pseudoxanthoma elasticum in the mid and lower dermis. An irregular but fairly sharply outlined nodular band of altered, often hyalinized, fibers or bundles was found in whorls or irregular streaks around particles or clumps of blue-staining material resembling calcium (Fig. 1). In the hematoxylin-and-eosin-stained sections most of the fibers resembled collagen. However, in the calcified areas there were numerous frayed, fragmented, or curled fibers which seemed to contain calcium as well as to be surrounded by it. The nature of these fibers could not be determined in the hematoxylin-and-eosin-stained sections, but they appeared different from the rather broad bands of connective tissue making up most of the nodule. Occasional macrophages were found in the calcified areas.

In the sections stained by the Lillie modification of the Masson trichrome stain most of the material in the nodular areas

Submitted for publication Nov. 22, 1957.

Aided in part by a grant (H-2560(C1)) from the National Heart Institute, National Institutes of Health, United States Public Health Service.

Departments of Pathology and Anatomy, University of Pittsburgh School of Medicine and the Presbyterian, Woman's, and Eye and Ear Hospitals.

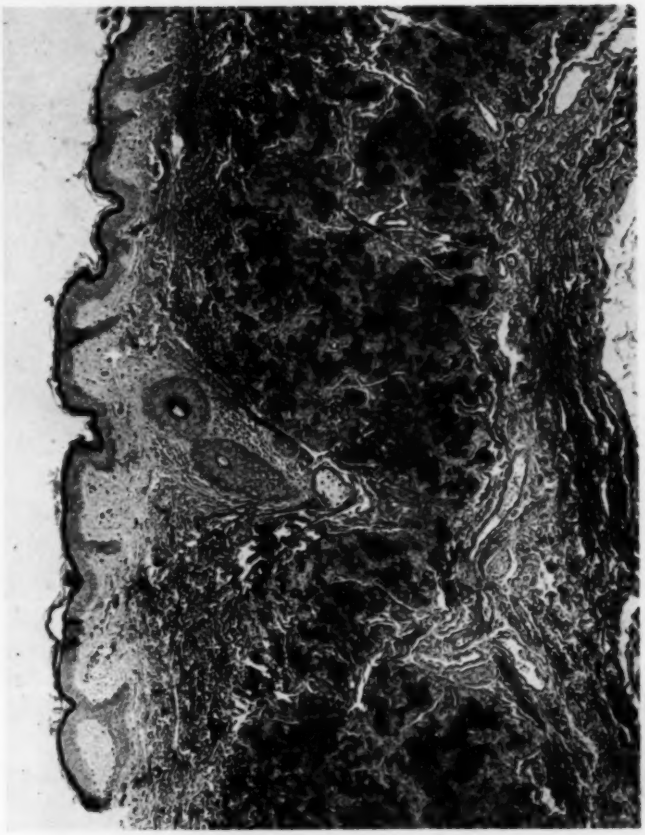

Fig. 1.—Photomicrograph showing characteristic involvement of mid and lower dermis, with dark-staining calcific deposits surrounded by lighter-staining collagen. Hematoxylin and eosin; × 210.

stained blue. The fibers were thick, often whorled, and dense, giving an impression of a definitely increased amount of collagen (Fig. 2). They surrounded fairly large masses of red-staining calcium, which again contained irregular short thick fibers. Some of these fibers stained red, and again the calcium appeared to be deposited in as well as around the fibers. In the sections stained by the Verhoeff elastic-tissue method, the elastic fibers in the upper dermis appeared normal. The irregular fibers in the calcified portions of the involved area were black, while the surrounding collagenous fibers were pink. At the lower margin of the involved area elastic tissue was prominent. The sections stained by the orcein method showed numerous varying-sized fragmented, often thick and irregular, red fibers which were indistinguishable from elastic tissue elements (Fig. 3). These were principally in the calcified portions, and they were surrounded by wide bundles of tissue resembling collagen.

Observation of the sections after digestion with elastase showed the characteristic change of elastic tissue. The fibers staining red with orcein assumed a prominent transversely segmented, striped, or ladder appearance as they underwent partial dissolution (45 minutes) (Figs. 4 and 5). At the end of two hours almost all of these fibers in the elastase-covered areas had disappeared, although a few thick fibers were still seen. These also showed a striped or segmented appearance. The involved fibers in areas protected from elastase action were unchanged, and the hyalinized collagen sur-

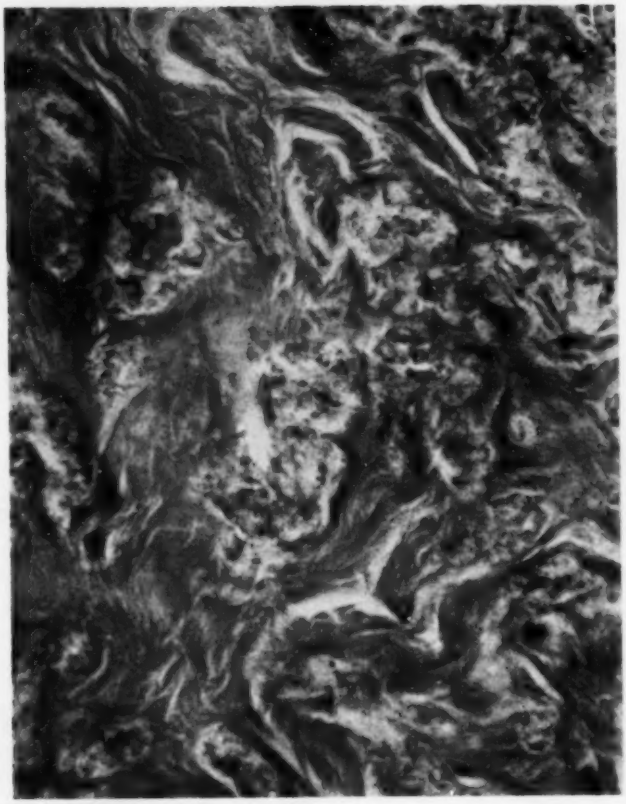

Fig. 2.—Photomicrograph showing whorled collagenous tissue bundles around the calcified areas. Masson trichrome; × 800.

Fig. 3.—The abnormal, curled, and thickened fibers. Orcein elastic tissue stain; × 800.

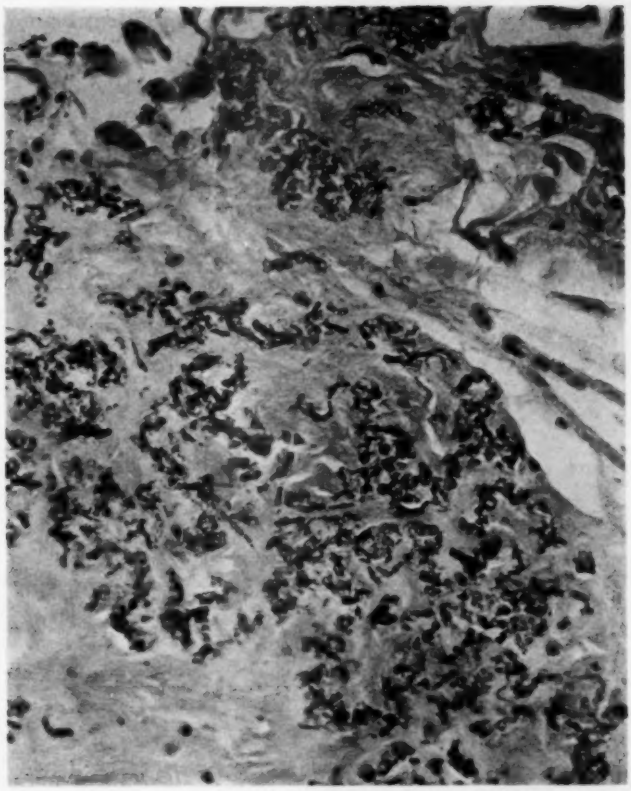

Fig. 4.—Low-power photomicrograph of involved area after elastase digestion for 45 minutes and orcein stain. The affected fibers stain darkly and many show transverse segmentation. The surrounding pale-staining collagen is not affected by the digestion. Orcein; × 45.

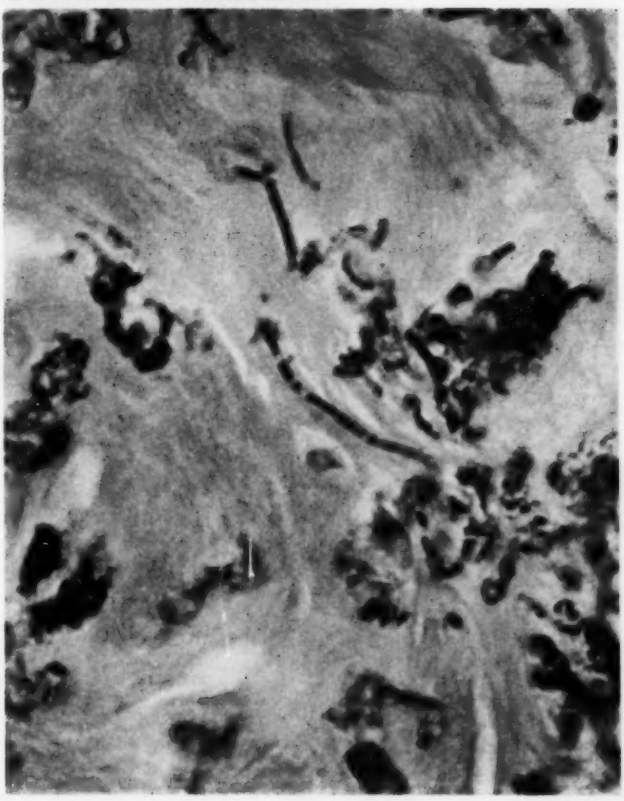

Fig. 5.—Higher magnification of a portion of the field in Figure 4, showing transverse segmentation of the elastase-digested fibers. Orcein; × 800.

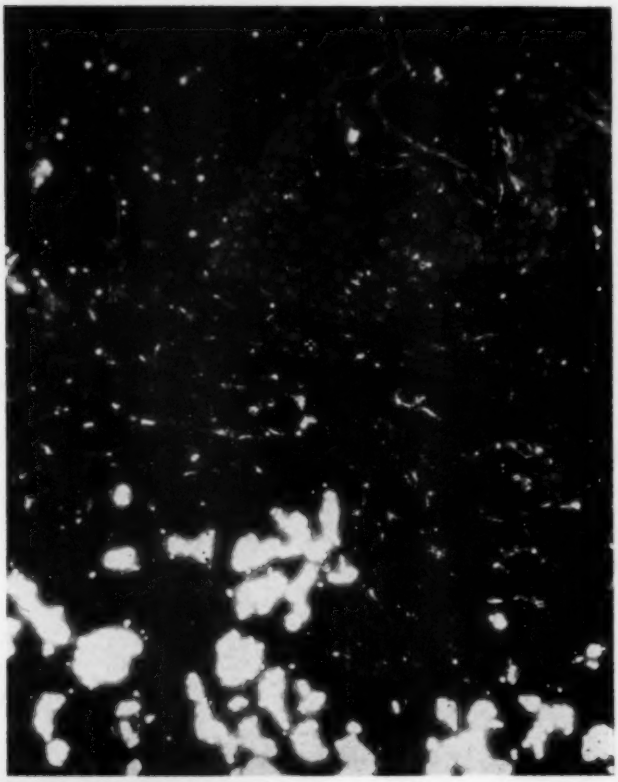

Fig. 6.—Dark-field photomicrograph after microincineration, showing the light-staining calcium masses in the dermis in contrast to the faintly outlined epidermis (upper); × 320.

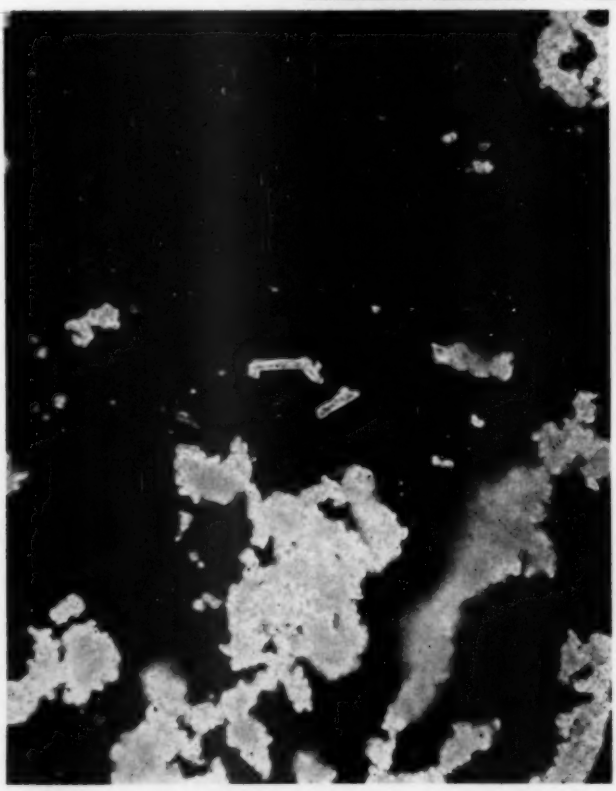

Fig. 7.—Two of the involved fibers under dark-field examination after microincineration. The fibers actively reflect light and appear to contain calcium particles; × 320.

Fig. 8.—Electron photomicrograph of collagen located in periphery of pseudoxanthoma elasticum lesion, showing both cross sections and longitudinal sections of fibrillae, the latter with transverse periodicity; reduced 20% from mag. approximately × 37,500.

rounding these fibers and the particles of calcium were not digested. Elastic tissue from normal skin and ligamentum nuchae exposed to elastase showed changes identical to those seen in the biopsy material. Control collagen fibers were unaltered.

Dark-field examination of the incinerated sections showed heavy deposits of the white ash characteristic of calcium and/or magnesium in the mid and lower dermis. This was in sharp contrast to the epidermis, which was delicately outlined with ash (Fig. 6). In the involved area of the dermis the white ash existed in irregularly dispersed large clumps. Occasional rod-shaped fibers, some sharply angulated, were found both in the large calcium clumps and adjacent to them. All of these discrete fibers were sharply outlined with the white ash and appeared to contain granules that actively reflected light in a manner indistinguishable from the large masses (Fig. 7).

In sections viewed by electron microscopy the appearance of the altered fibers was typical of elastic tissue and did not resemble collagenous tissue. These fibers were surrounded by and mixed with fibers with the 640 A. periodicity typical of collagen (Figs. 8 and 9).

## Comment

Although Balzer [3] originally described the skin disease in 1884 and Darier [4] described and named the condition in 1896, wide recognition of the generalized nature of pseudoxanthoma elasticum came after Grönblad [5] described its association with angioid retinal streaks in 1929. Descriptions of patients with hypertension, visceral and peripheral arterial involvement, and gastrointestinal hemorrhage have further strengthened the concept of a generalized disease with skin involvement constituting only one of several principal components. McKusick [6] recently summarized much of the current knowledge of the disease and provided a comprehensive bibliography.

The classical histologic changes described in the skin include degeneration of elastic (or collagenous) fibers in the mid and lower dermis, with basophilic staining, whorling, curling, shortening, thickening, and fragmentation of the fibers. Calcium deposition is frequent and in late stages may overshadow the rest of the histologic picture. The epidermis and the upper dermis usually appear normal.

The majority opinion for many years held that pseudoxanthoma elasticum is a disease of elastic tissue, and studies by Findlay [7] with elastase have recently strengthened this viewpoint. Findlay demonstrated that elastase dissolved the elastic tissue around the pathological nodules and

Fig. 9.—Electron photomicrograph of area immediately adjacent to that shown in Figure 8. The difference in electron density and internal structure between a collagen fiber (lower left) and four amorphous elastic fibers is apparent; approximately × 30,000.

the material within the nodules. The degenerated material within the nodules showed slightly greater resistance to elastase than normal elastic fibers.

However, other observers, including Hannay [8] and Tunbridge and collaborators,[9] have presented evidence suggesting that the affected material is collagen. McKusick [6] has summarized the arguments in favor of the idea that pseudoxanthoma elasticum is primarily a disease of collagen. However, several of the points listed by McKusick can be refuted in the light of present knowledge.

McKusick refers to Findlay's digestion of the involved fibers with elastase, but he states that elastase digestion is not specific and quotes Hall, Reed, and Tunbridge [10] to the effect that elastase is fundamentally a mucase and Banga [11] that elastase has effect on normal collagen. Since these observations, elastase has been purified, and it has now been demonstrated [12-15] that elastase digestion is relatively specific for elastic tissue. Furthermore, the transverse segmentation or ladder appearance of the elastic fibers under digestion and before complete disappearance is characteristic of

elastic fibers and does not occur with native collagen.[1]

Another point quoted by McKusick favoring the role of collagen in this disease is that the skin normally contains relatively few elastic fibers and that these are concentrated immediately beneath the epidermis. This point may be answered in several ways. First, part of the statement is incorrect, since many of the elastic fibers in normal skin are concentrated in the mid and lower dermis. Second, there is a possibility that elastic tissue may proliferate in this disease or it may simply stand out more prominently because of the degenerative changes. Third, and probably most important, our observations indicate that much of the connective tissue in the involved area is actually collagen. However, the fibers in dispute, those in the areas of calcification, have the characteristics of elastic tissue. The amount of this tissue is entirely in keeping with the amount of elastic tissue in normal skin. These fibers also appear to contain calcium, as well as being surrounded by it. This is a common feature of elastic fibers, while it is our impression that deposition of calcium within collagenous fibers does not ordinarily occur. The presence of macrophages and giant cells in this disease [16,17] suggests that the involved fibers may act to some extent as foreign material. Foreign-body giant-cell reaction and diffuse interstitial fibrosis in response to altered elastic fibers in the lung have been described by Walford and Kaplan.[18] Increase in the collagen in the involved area of skin in pseudoxanthoma elasticum would seem a logical sequence of the elastic tissue degeneration.

McKusick also cites the involvement of muscular arteries in this disease and the lack of specific changes in the tunica elastica interna in these vessels as evidence against the "elastic tissue" theory. This would not appear as valid evidence in favor of primary collagen involvement, as many other factors could contribute to early development of arteriosclerosis in these patients. The work of Tunbridge and his collaborators [9] with electron microscopy is quoted to the effect that the involved fibers have the characteristic 640 A. periodicity of collagen. Our studies by electron microscopy clearly show the elastic tissue fibers without internal organization often surrounded by fibers with characteristic collagen periodicity. It would be easy to conclude that all of the fibers were collagen if the fields examined were not selected carefully, with orientation of the fields under phase microscopy.

In a previous report [1] the specific nature of the alterations in elastic tissue under elastase digestion and the appearance of elastic tissue under electron microscopy have been described. In the present study we have demonstrated elastase digestion of the abnormal fibers, the transverse segmentation of these fibers occurring during digestion, the presence of calcium in the involved fibers by microincineration, and the lack of the characteristic collagen periodicity of these fibers under electron microscopy. On the basis of these specific changes we believe that the fibers in pseudoxanthoma elasticum which have an affinity for the established elastic tissue stains and which resemble elastic tissue in hematoxylin and eosin stains are actually elastic fibers. Quantitative estimations of the relative amounts of elastic tissue and collagen were not attempted in this study, but the relative proportions may be seen in the low-power photomicrograph stained by orcein (Fig. 4) in which the elastic fibers are dark and the collagen, pale. We suggest that these altered elastic fibers may act as foreign material, evoking an inflammatory and giant-cell reaction, calcification, and eventually an increase in collagenous fibrous tissue in the involved portions of skin. Our results do not exclude the possibility that both elastic and collagenous fibers are primarily involved in this disease, although this seems much less likely.

## Summary

Portions of involved skin from a typical clinical and histologic example of pseudoxanthoma elasticum have been studied by standard histologic methods, elastase digestion, microincineration, and electron microscopy. The results of these studies strongly support the viewpoint that the primarily altered fibers in this disease are derived from elastic tissue. There is also a definite increase in collagenous tissue in the involved areas. This is regarded as a secondary change arising in response to the primary elastic tissue alteration. This concept of the pathogenesis of the disease resolves some of the discrepancies previously reported on the nature of the involved fibers in pseudoxanthoma elasticum.


Presbyterian Hospital, 230 Lothrop St. (13).

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# News and Comment

## PERSONAL

**Award to Dr. Jacob Furth.**—The 1958 Bertner Foundation Award for outstanding contribution to the field of cancer research was presented on March 7, 1958, to Dr. Jacob Furth, Associate Director of Research, Children's Cancer Research Foundation, Boston. He was presented with a bronze medallion symbolizing the goal of cancer research—prevention as well as cure. Dr. Furth lectured on the subject "Radiation Neoplasia and Endocrine Systems."

**Scholarship Awarded to Dr. Robert B. Jennings.**—Dr. Robert B. Jennings, Assistant Professor of Pathology, Northwestern University Graduate and Medical Schools, was awarded one of the 25 scholarships in Medical Science of The John and Mary R. Markle Foundation. This scholarship is for five years.

**Paper Presented by Dr. Edward A. Gall.**—Dr. Edward A. Gall presented a paper before the Annual Clinical Conference of the Chicago Medical Society on March 4, 1958. His title was "Cancer as a Multicentric Lesion."

**Dr. Shields Warren Honored by Dinner.**—Dr. Shields Warren, of Boston, was honored by a dinner at the Hotel Statler on Feb. 26. Friends and colleagues paid tribute to him for his distinguished contributions to science and mankind.

**Award to Sir Howard Florey.**—Sir Howard Florey, Professor of Pathology at the University of Oxford, was recently awarded the Copley Medal of the Royal Society of London on the occasion of the 295th anniversary of the Society.

**Dr. W. D. Stovall Retires.**—Dr. W. D. Stovall is retiring on July 1, 1958, from his position as Director of the Clinical Laboratories of University Hospitals and Director of the program of Clinical Pathology in the University of Wisconsin Medical School. His positions are to be taken by Dr. Frank C. Larson, associate professor of medicine at the University of Wisconsin Medical School.

## ANNOUNCEMENTS

**Meeting of Inter-Society Cytology Council.**—The Annual Scientific Meeting of the Inter-Society Cytology Council will be held at the Hotel Statler, New York, Nov. 13, 14, and 15, 1958. For further details write to the Secretary, Dr. Paul F. Fletcher, 634 N. Grand Ave., St. Louis 3.

**Law-Medicine Research Institute.**—A Law-Medicine Research Institute, the first of its kind in the United States, has been established at Boston University. The Institute, working cooperatively with the University schools of law and medicine, is to be headed by Deans Elwood H. Hettrick and Chester S. Keefer. The plans are to establish a program of research and training in the interdisciplinary areas of law, medicine, and the behavioral sciences and to act as a clearing-house for information on activities in the medicolegal area.

**Postgraduate Courses.**—Announcement is made of the following postgraduate courses of interest to pathologists:

*Title.*—"The Principles and Techniques of Tissue Culture."
*Place.*—University of Colorado School of Medicine, Denver.
*Dates.*—July 7 to August 1, 1958.
*Fees.*—$100.
*Address for Inquiries.*—Dr. Mary S. Parshley, College of Physicians and Surgeons, 630 W. 168th St., New York 32.

*Title.*—"Histochemistry."
*Place.*—University of Kansas Medical Center, Kansas City, Kan.
*Dates.*—June 9 to 21, 1958.

*Fees.*—One week, $75; two weeks, $125.

*Address for Inquiries.*—Department of Postgraduate Medical Education, University of Kansas School of Medicine, Kansas City 12, Kan.

**Gordon Research Conferences.**—The Gordon Research Conferences for 1958 will be held from June 9 to Aug. 29 at Colby Junior College, New London, N. H.; New Hampton School, New Hampton, N. H., and Kimball Union Academy, Meriden, N. H.

The Conferences were established to stimulate research in universities, research foundations, and industrial laboratories. This purpose is achieved by an informal type of meeting consisting of scheduled lectures and free discussion groups. Sufficient time is available to stimulate informal discussions among the members of a Conference. Meetings are held in the morning and in the evening, Monday through Friday, with the exception of Friday evening. The afternoons are available for participation in discussion groups as the individual desires. This type of meeting is a valuable means of disseminating information and ideas which otherwise would not be realized through the normal channels of publication and scientific meetings. In addition, scientists in related fields become acquainted and valuable associations are formed which result in collaboration and cooperative effort between different laboratories.

It is hoped that each Conference will extend the frontiers of science by fostering a free and informal exchange of ideas between persons actively interested in the subjects under discussion. The purpose of the program is not to review the known fields of chemistry but primarily to bring experts up to date as to the latest developments, to analyze the significance of these developments, and to provoke suggestions as to underlying theories and profitable methods of approach for making new progress. In order to protect individual rights and to promote discussion, it is an established rule of each Conference that all information presented is not to be used without specific authorization of the person making the contribution, whether in formal presentation or in discussion. No publications are prepared as emanating from the Conferences.

Persons interested in attending a Conference are requested to send their applications to the Director. Each applicant must state the institution or company with which he is connected and the type of work in which he is most interested. Attendance at each Conference is limited to 100.

The complete program of the Conferences will be published in *Science* for Feb. 28.

Requests for attendance at the Conferences, or for any additional information, should be addressed to W. George Parks, Director, Department of Chemistry, University of Rhode Island, Kingston, R. I. From June 9 to Aug. 29, 1958, mail should be addressed to Colby Junior College, New London, N. H.

Cancer—H. G. Schlumberger, Chairman, Frederik B. Bang, Vice-Chairman

Aug. 25

J. J. Biesele: Early Cytological Effects of Carcinogens
Stanfield Rogers: Mechanism of Urethane-Induced Lung Carcinoma in Mice
Wilhelm Hueper: Carcinogenic Studies on Water Soluble and Insoluble Macromolecules
S. C. Sommers: Pathological Evidence of Endocrine Imbalance in Cancer Patients
J. Leighton: Mechanisms of Tumor Invasion as Seen in Tissue Culture

Aug. 26

L. Kilham: Fibroma of Squirrels Transmitted by a Virus Immunologically Related to the Shope Rabbit Fibroma Virus
R. C. Mellors: Fluorescent Antibody Study of Viruses in Experimental Neoplasms
J. A. Reyniers: Germ-Free Animals and Cancer Research
Alfred Gellhorn, Chairman
Abraham Goldin: Competitive Analogue-Metabolite Relationships in Cancer Chemotherapy
Julian J. Jaffe: The Chemotherapy of Neoplasm Using Azaurocil and Azauridine
J. F. Holland: Tumor Suppressive Activity in Tumor Ascites Fluid

Aug. 27

E. Zwilling: Differentiation of Early Chick Embryo Tissues Following Disaggregation and Reaggregation

D. McKay: Histochemistry of the Migrating Primordial Germ Cell
G. Barry Pierce: Tissue- and Organogenesis in Mouse Teratomas
H. S. Fleming: Organoid Growth of Transplants in the Eye
W. F. Loomis: The Role of $CO_2$ Tension in Cell Differentiation (Hydra)

Aug. 28

E. C. Hammond: Epidemiological Methods in Cancer Research
Lalla Iverson: Incidence of Chorionepithelioma in South-East Asia
J. Higginson: Cancer in the South African Bantu
E. S. Wynne: Epidemiology of "Cancer Eye" in Cattle
H. Innes: Divergent Susceptibilities of Animal Species to Different Types of Cancer

Aug. 29

Henry Plaine, Chairman
A. E. Kehr: Genetic Tumors in Plants (Nicotiana)
Madge T. Macklin: Heredity in Human Cancer
F. Friedman and L. Burton: Etiology of Tumors in Drosophila

## DEATHS

**Dr. Ernest Kennaway.**—Dr. Ernest Kennaway, London, England, professor emeritus of experimental pathology at the University of London, died on Jan. 1, 1958.

**Dr. Alfred S. Giordano.**—Dr. Alfred S. Giordano of South Bend, Ind., died in Sarasota, Fla., on Feb. 15, 1958.

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# Books

**Histologie der Hautkrankheiten: Vol. 1. Dermatitiden I. Normale Anatomie und Entwicklungsgeschichte, Leichenerscheinungen, Dermatopathien. Vol. 2. Dermatitiden II. Oertlich übertragbare infektiöse Gewebsneubildungen: Tierische Parasiten und Fremdkörper; Störungen des Kreislaufs; Entwicklungsstörungen; Echte Geschwülste.** By O. Gans and G. K. Steigleder. Price, DM 285. Pp. 726, with 258 illustrations. Springer-Verlag, Reichpietschufer 20, (1) Berlin W. 35, 1955.

Since his early years of development, Dr. O. Gans, Professor of Dermatology at the University of Frankfurt, has been a highly successful clinician and investigator. He was the first to apply the Warburg respirometer to normal and pathological skin tissues and the first to perform blood analyses in skin diseases. However, his name became known all over the world only after he published his monumental two-volume "Histology of Skin Diseases." The first volume appeared in 1925, and the second, in 1928. This was the first modern book on the subject, actually the first since the publication of Unna's "Histopathology of Skin Diseases," in 1894. Gans' book had a well deserved acclaim. He became well known and appreciated in this country. Many of our most prominent dermatohistopathologists have been his disciples.

The book was out of print for many years. It is most gratifying that a second edition has now been published. Dr. Steigleder, one of the most talented representatives of cutaneous histopathology and histochemistry of the younger generation, is co-author of this second edition. The book has been brought up to date in all respects. For instance, there is a new section on dermatomyositis; the systemic nature of pseudoxanthoma elasticum is stressed; the new literature on the acantholytic reaction in pemphigus is critically discussed; the chapter on pathology of deep mycoses (including histoplasmosis) is newly written, and eosinophilic granuloma of the face is discussed. As in the first edition, each entity is succinctly introduced by a short paragraph on the nature of the disease. While the authors conscientiously quote the pertinent literature, their individual critical point of view is reflected through the work.

The organization of the material is good. The main headings are as follows: Normal Anatomy and Embryology, Post-Mortem Changes, Atrophies, Dystrophies, Dermatitides (Reactive Processes), Infectious Neoplasms, Animal Parasites and Foreign Bodies, Circulatory Disorders, Developmental Anomalies, True Neoplasms.

An interesting feature of the work is its illustrations. In contrast to the modern trend, most of the 258 pictures are colored drawings by a first-class artist. These drawings are so excellent and true that the reader will at least partially agree with the authors that this technique has certain advantages over photomicrography.

The work of Gans and Steigleder should be on the shelf of everyone who deals with problems of dermatohistopathology.

While the first edition was dedicated to the old master, P. G. Unna, this second edition is appropriately dedicated to the predecessor of Gans at Frankfurt, Karl Herxheimer, a great clinician and a fine gentleman who, broken at the age of 82 by a cruel barbaric government, died in a Nazi concentration camp in 1942.

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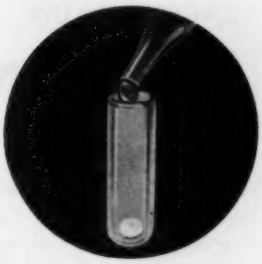

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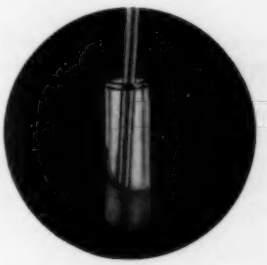

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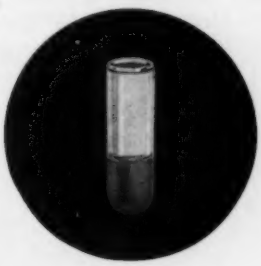

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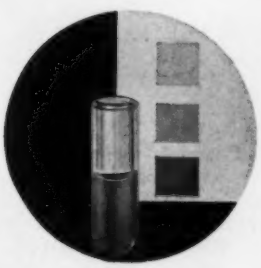

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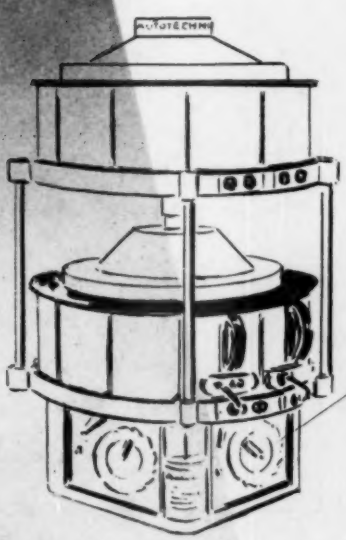

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